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I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2003906633 for a patent by THE UNIVERSITY OF QUEENSLAND as filed on 28 November 2003.

WITNESS my hand this
Fourteenth day of December 2004

LEANNE MYNOTT
MANAGER EXAMINATION SUPPORT
AND SALES

AUSTRALIA
Patents Act 1990

PROVISIONAL SPECIFICATION

Invention Title: ANTI-CANCER AGENTS

Applicant: THE UNIVERSITY OF QUEENSLAND

The invention is described in the following statement:

Anti-Cancer Agents

Field of the Invention

The present invention relates to anti-cancer agents, particularly anti-cancer agents that have a core framework structurally related to or derived from cysteine and which may be utilised in cancer therapies either on their own or in combination with other anti-cancer agents. The invention further provides pharmaceutical and/or veterinary compositions containing the anti-cancer agents of the invention that may be used in the treatment of cancers. The invention further relates to the use of the anti-cancer agents of the invention in the preparation of medicaments for the treatment of cancer and to methods of treatment of cancer using the anti-cancer agents or compositions containing them.

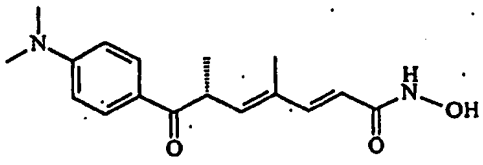
Background of the Invention

Cancer is one of the leading causes of death in the modern world with the incidence of cancer related deaths rising with the ageing population. Many other causes of death that were prevalent at the beginning of the 20th century have been eliminated over the last 100 years due to advances in modern medicine. It is expected that further advances in pharmaceutical science will eliminate further conditions that cause premature mortality therefore leading to increased life expectancy which in turn will lead to a proportional increase in the number of deaths from cancer.

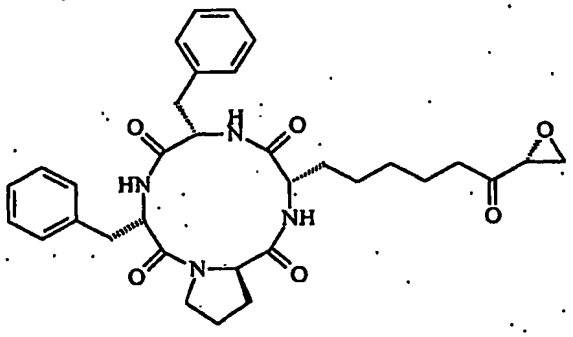
At the present time there are three main treatment strategies for cancer: (1) removal of the cancer by surgery (where possible), (2) use of radiotherapy, or (3) use of combination chemotherapy. With some cancer types a combination strategy is used in which as much of the cancerous tissue being removed by surgery as possible followed by a course or courses in chemotherapy to eliminate any remaining cancer cells. The exact method of choice for cancer treatment is typically determined on a case by case basis depending on the type of cancer and the extent that the cancer has spread from the original site of

cancer growth. A major dose-limiting problem associated with most chemotherapy is the general toxicity of the drugs currently available. Anti-cancer drugs today are typically general cytotoxins without much selectivity in their killing action for cancer cells over normal human cell types. This lack of selectivity leads to a significant number of adverse side effects in patients who undergo chemotherapy.

The development of truly selective cancer chemotherapy in which a drug specifically destroys malignant cells without damaging normal cells remains an elusive goal. One promising strategy is the use of agents that can differentiate cancer cells to either a non-proliferating or normal phenotype, an approach that has the potential to be tissue-specific and avoid side effects of current drugs. However, most compounds known to differentiate tumour cells are of low potency in cell culture and tend to be non-selective *in vivo*, where differentiation is reversible or drug resistance is a problem. A few natural products (e.g. trichostatins, trapoxins) and close analogues display more potent differentiating properties on tumour cells *in vitro*, but they are cytotoxic to both normal and cancer cells and are ineffective *in vivo* due to low bioavailability and rapid metabolism. Representative of the structural formulae of these compounds are Trichostatin A and Trapoxin B as shown below..



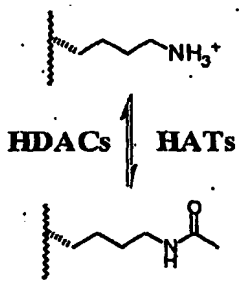
Trichostatin A



Trapoxin B

All of the differentiating agents discussed above are now known to cause hyperacetylation of histones, by inhibiting enzymes known as histone deacetylases (HDACs). It is also clear that multi-protein complexes incorporating

HDACs are involved in cell cycle regulation and gene expression. HDACs are involved in modulating chromatin structure by facilitating unpackaging of chromosomal DNA and 'loosening up' histones to permit transcription. Histones of the nucleosome are normally tightly wrapped in DNA and linked together, like a string of beads by DNA. Nuclease-mediated digestion of both the linking and wrapping DNA from histones enables gene expression. Unwrapping exposes the octameric histone core, which dissociates into component histones H2A, H2B, H3, H4, etc. Histones are reversibly acetylated on the ε-amino side chain of Lys residues as shown below, and interactions between *deacetylated* histones and DNA are crucial for gene expression. Histone acetylation and other modifications regulate gene expression by reducing access of transcription factors to DNA. The degree of histone acetylation is regulated by histone acetyl transferases (HATs; 3 groups), deacetylases (HDACs, 16 genes), and their inhibitors, which regulate the cell cycle and consequently hold promise for development of anticancer drugs. Studies by the current applicants and others indicate that HDAC inhibitors cause tumor regression *in vivo* without damaging DNA.



At least eleven HDACs have been identified and, although it is unknown to what extent these enzymes exercise redundant or specific functions, subtle sequence differences between HDACs suggest that it may be possible to develop inhibitors that are selective for specific HDAC enzymes. Crystallographic studies on the histone deacetylase-like protein (HDLP) isolated from *Aquifex aeolicus* indicate that the active site residues of these enzymes are highly conserved, with more variability at the entrance to this cleft, particularly on the solvent exposed rim of the active site that accommodates the lysine side chain. Furumai et al. has shown that a carboxylic acid analogue of trapoxin, which is a

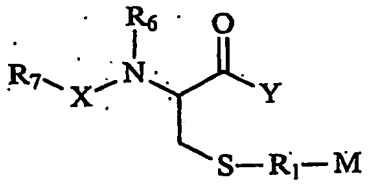
poorer zinc ligand, is still potent with IC_{50} of 100 nM probably due to the existence of significant interactions with the protein surface at the entrance to the HDAC active site.

Notwithstanding the potential of the above compounds and analogues thereof as anti-cancer agents, there still exists the need to develop further potential anti-cancer agents that provide viable alternatives to the known treatments. In particular there is the need to develop anti-cancer agents that have therapeutic efficacy *in vivo* and which show some degree of selectivity for cancer cells.

The present applicants have therefore conducted investigations into the mode of action of the compounds discussed above with a view to developing alternative cancer treatment agents.

Summary of the Invention

The present invention provides a compound having the formula (1), or a pharmaceutically acceptable derivative, salt, racemate, isomer or tautomer thereof:



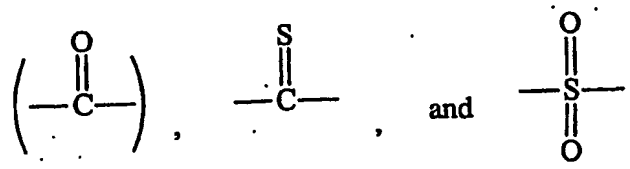
(1)

R_1 is a linking moiety;

M is a zinc binding moiety containing at least one heteroatom;

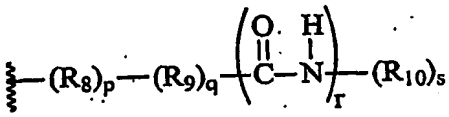
R_6 is selected from the group consisting of H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl and a nitrogen protecting group,

X is selected from the group consisting of



Y is selected from the group consisting of $-NR_4R_5$, $-OR_4$, $-SR_4$, $-CH_2R_4$, CHR_4R_5, $C(R_4)_2R_5$, PHR_4 and PR_4R_5,

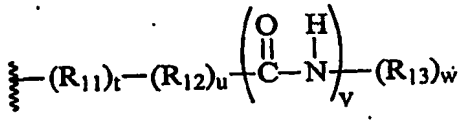
wherein R_4 is a group of formula:



wherein R_8, R_9 and R_{10} are each independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, and optionally substituted heterocycloalkyl;

p, q, r and s are each independently 0 or 1, provided that at least one of p, q or s is 1;

R_5 is H or a group of formula:



wherein R_{11}, R_{12} and R_{113} are each independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, or optionally substituted heterocycloalkyl;

t, u, v and w are each independently 0 or 1, provided that at least one of t, u and w is 1;

R_7 is a group of formula:

$$(R_{16})_z\text{-}(R_{15})_y\text{-}(R_{14})_x\text{—}$$

wherein R_{14}, R_{15} and R_{16} are independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl and optionally substituted heterocycloalkyl;

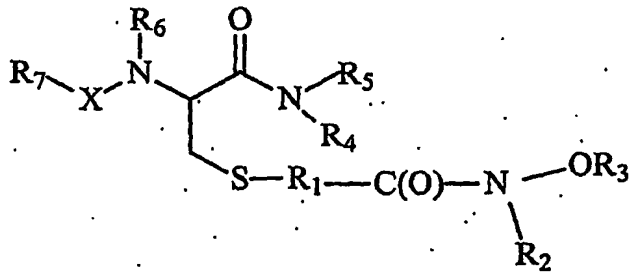
x, y and z are independently 0 and 1 with the proviso that at least one of x, y and z is 1.

As with all chemical families there are a number of preferred embodiments within the scope of the general formula. In particular it is preferred that the linker has between 1-9 atoms in the normal chain, preferably between 1 and 4 atoms in the normal chain.

It is also preferred that the group Y is a group of formula $-NR_4R_5$.

It is preferred that the zinc binding moiety containing a heteroatom is a hydroxamic acid derivative, preferably a group of formula $-C(O)-NR_2-OR_3$ where R_2 is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, or a nitrogen protecting group and R_3 is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl or an oxygen protecting group;

Accordingly in a preferred embodiment the present invention provides a compound having the formula (2), or a pharmaceutically acceptable derivative, salt, racemate, isomer or tautomer thereof:



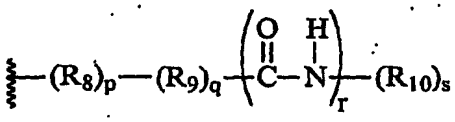
(2)

R_1 is optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 alkenyl or optionally substituted C_1-C_4 alkynyl;

R_2 is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, or a nitrogen protecting group;

R_3 is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl or an oxygen protecting group;

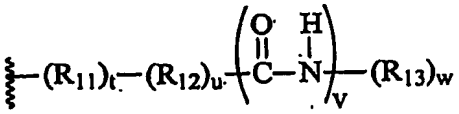
R_4 is a group of formula:



wherein R_8, R_9 and R_{10} are each independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, and optionally substituted heterocycloalkyl;

p, q, r and s are each independently 0 or 1, provided that at least one of p, q or s is 1;

R_5 is H or a group of formula:

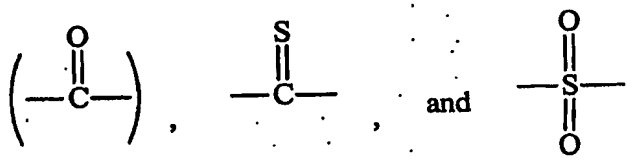


wherein R_{11}, R_{12} and R_{113} are each independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, or optionally substituted heterocycloalkyl;

t, u, v and w are each independently 0 or 1, provided that at least one of t, u and w is 1.

R_6 is selected from the group consisting of H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl and a nitrogen protecting group,

X is selected from the group consisting of



R_7 is a group of formula:

$$(R_{16})_z\text{-}(R_{15})_y\text{-}(R_{14})_x—$$

wherein R_{14}, R_{15} and R_{16} are independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl and optionally substituted heterocycloalkyl,

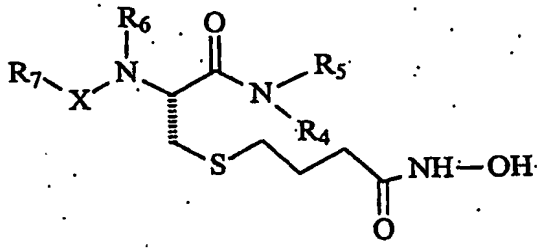
x, y and z are independently 0 and 1 with the proviso that at least one of x, y and z is 1.

Even within this preferred subset of compounds there are a number of preferred values for each of the variables in the structural formula given above. For example it is preferred that R_1 is optionally substituted C_1-C_4 alkyl, more preferably optionally substituted C_2-C_3 alkyl, even more preferably optionally substituted C_3 alkyl, most preferably propyl.

It is preferred that R_2 is either H, optionally substituted C_1-C_4 alkyl or a nitrogen protecting group, more preferably H or a nitrogen protecting group, most preferably H.

It is preferred that R_3 is either H, optionally substituted C_1-C_4 alkyl or an oxygen protecting group, more preferably H or an oxygen protecting group, most preferably H.

Particularly preferred compounds of formula (2) are therefore those of formula (2a).



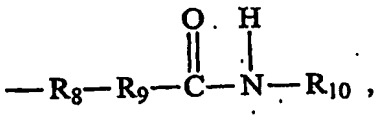
(2a)

In the compounds of the invention it is preferred that X is a carbonyl group.

It is preferred that R_5 is either H or optionally substituted alkyl, preferably H.

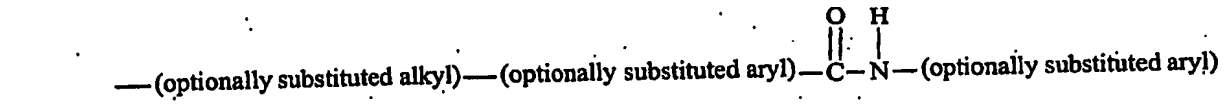
It is preferred that R_6 is either H or a nitrogen protecting group, most preferably H.

In one preferred embodiment the group R_4 is of the formula

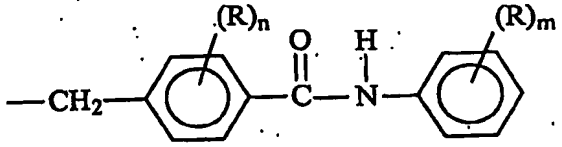


wherein R_8, R_9 and R_{10} are as defined above.

In this embodiment it is particularly preferred that R_4 is of the formula:



In the most preferred form of this embodiment R_4 is a group of the formula.



wherein each R is independently selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, haloheteroaryl, halocycloalkyl, haloheterocycloalkyl, hydroxy, alkoxy, alkenyloxy, aryloxy, heteroaryloxy, cycloalkyloxy, heterocycloalkyloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, halohetoraryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheteroaryl, nitroheterocyclyoalkyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, heteroarylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, heteroarylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphonyloxy, heterocycloalkylamino, alkylsulphonyl, arylsulphonyl, carboalkoxy, carboaryloxy, alkylthio, benzylthio, acylthio, cyano, nitro, sulfate and phosphate;

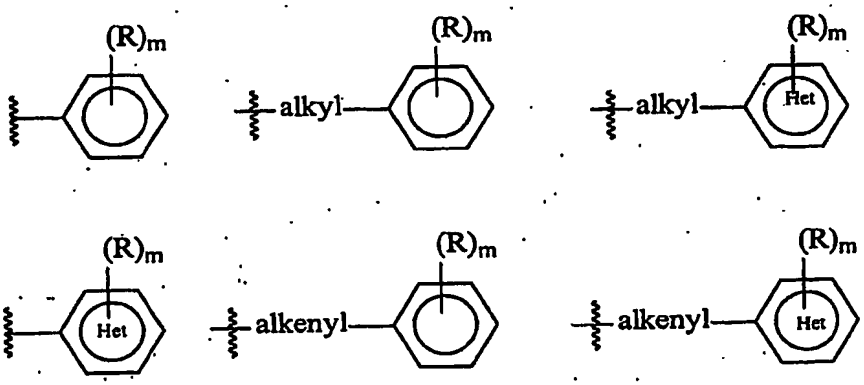
n is 0-4, and

m is 0-5.

In an another preferred embodiment of the invention R_4 is selected from the group consisting of: optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted cycloalkyl, optionally substituted heteroaryl, optionally substituted heterocycloalkyl, optionally substituted arylalkyl, optionally substituted heteroarylalkyl, optionally substituted cycloalkylalkyl, optionally substituted heterocycloalkylalkyl, optionally substituted aryl alkenyl, optionally substituted heteroaryl alkenyl, optionally substituted cycloalkyl alkenyl, optionally substituted heterocycloalkyl alkenyl, optionally substituted aryl alkynyl; optionally substituted heteroaryl alkynyl optionally substituted cycloalkyl alkynyl, optionally substituted heterocycloalkyl alkynyl.

In this embodiment it is particularly preferred that R_4 is selected from optionally substituted aryl, optionally substituted cycloalkyl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted arylalkyl, optionally substituted heteroarylalkyl, optionally substituted cycloalkyl alkyl, optionally substituted alkyl aryl, optionally substituted alkyl heteroaryl, optionally substituted alkyl heterocycloalkyl.

In a most preferred embodiment of the invention R_4 has one of the following Formulas.



Wherein each R is independently selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, haloheteroaryl, halocycloalkyl,

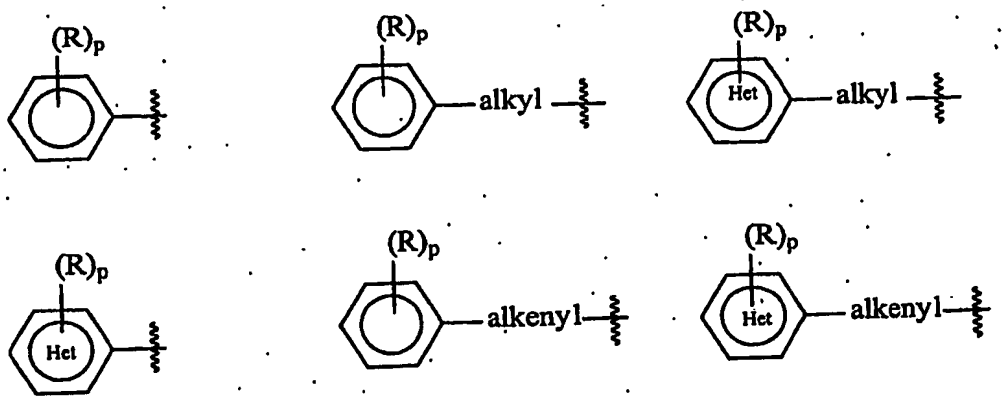
haloheterocycloalkyl, hydroxy, alkoxy, alkenyloxy, aryloxy, heteroaryloxy, cycloalkyloxy, heterocycloalkyloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, halohetoraryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheteroaryl, nitroheterocyclyoalkyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, heteroarylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl,heteroarylacyl, acylamino, diacylamino, acyloxy, alklysulphonlyoxy, arylsulphonyloxy, heterocycloalkylamino, alkylsulphonyl, arylsulphnyl, carboalkoxy, carboaryloxy, alkylthio, benzylthio, acylthio, cyano, nitro, sulfate and phosphate;

and each m is from 0-5.

In the compounds of the invention it is preferred that R_7 is selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted cycloalkyl, optionally substituted heteroaryl, optionally substituted heterocycloalkyl, optionally substituted aryl alkyl, optionally substituted heteroaryl alkyl, optionally substituted cycloalkyl alkyl, optionally substituted heterocycloalkyl alkyl, optionally substituted aryl alkenyl, optionally substituted hetero alkenyl, optionally substituted cycloalkyl alkenyl, optionally substituted heterocycloalkyl alkenyl, optionally substituted aryl alkynyl, optionally substituted heteroaryl alkynyl, optionally substituted cycloalkyl alkynyl, and optionally substituted heterocycloalkyl alkynyl.

It is even more preferred that R_7 is optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl alkyl, optionally substituted alkenyl, and optionally substituted aryl alkenyl.

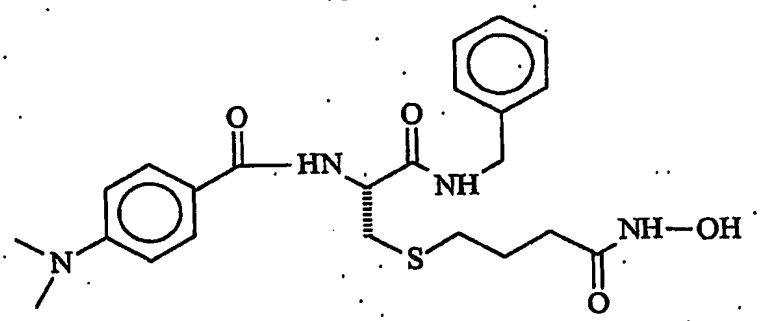
It is most preferred that R_7 has one of the following formula:

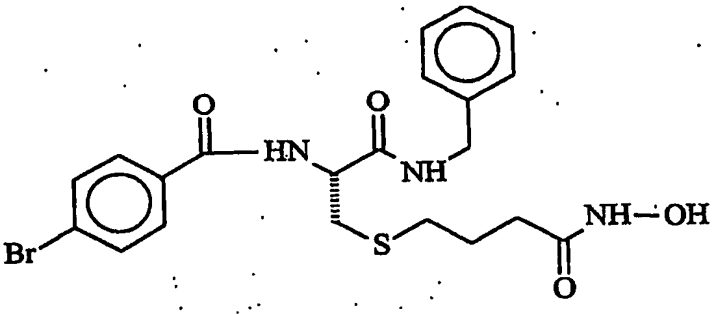


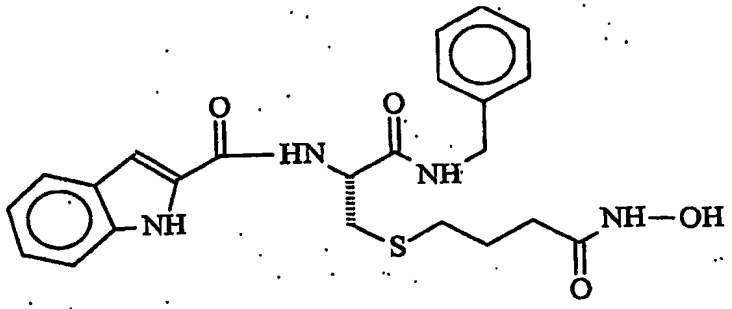
Wherein each R is independently selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, haloheteroaryl, halocycloalkyl, haloheterocycloalkyl, hydroxy, alkoxy, alkenyloxy, aryloxy, heteroaryloxy, cycloalkyloxy, heterocycloalkyloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, halohetoraryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheteroaryl, nitroheterocyclyoalkyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, heteroarylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl,heteroarylacyl, acylamino, diacylamino, acyloxy, alklysulphonlyoxy, arylsulphonyloxy, heterocycloalkylamino, alkylsulphonyl, arylsulphonyl, carboalkoxy, carboaryloxy, alkylthio, benzylthio, acylthio, cyano, nitro, sulfate and phosphate;

and each p is from 0-5.

A number of specific compounds are particularly preferred. These include the following.







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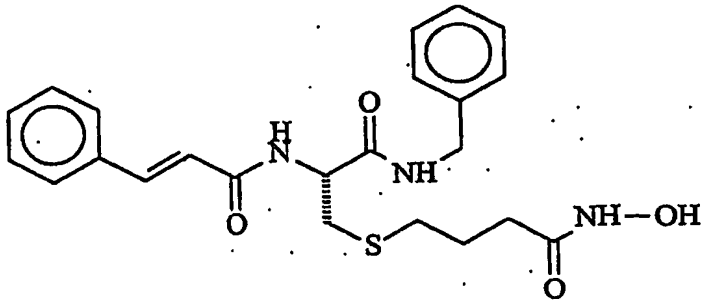

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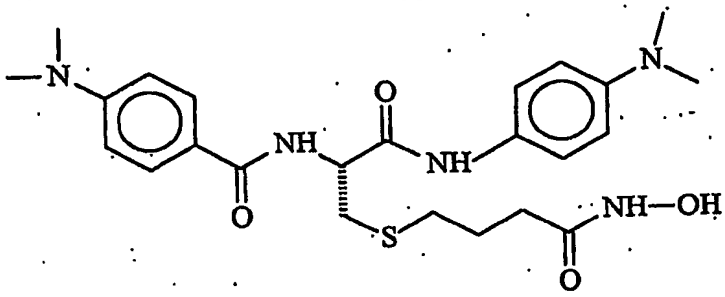
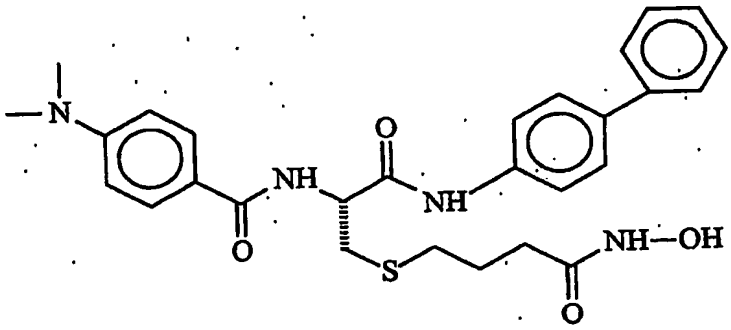
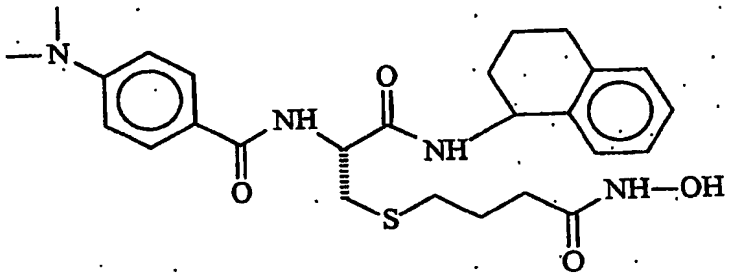
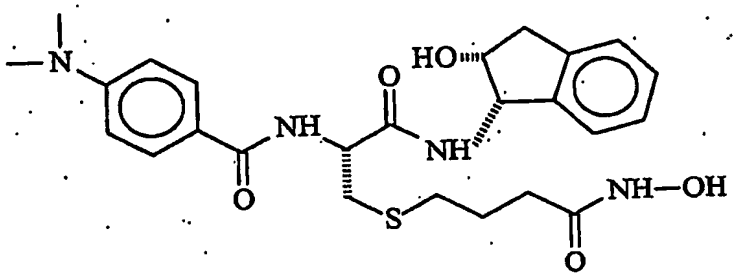
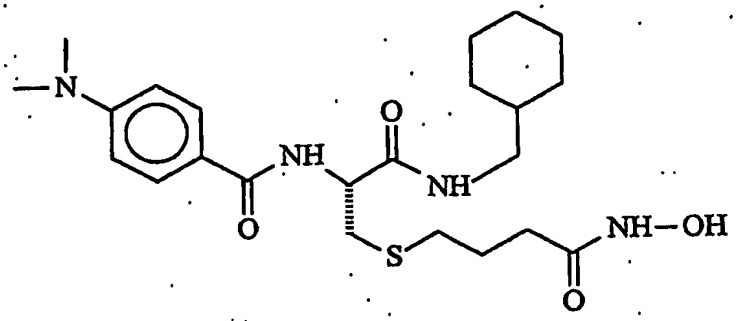




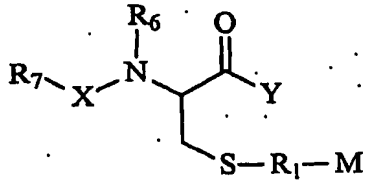






All stereoisomers (for example, geometric isomers, optical isomers and the like) of the present compounds (including those of the salts, solvates and prodrugs of the compounds as well as the salts and solvates of the prodrugs), such as those which may exist due to asymmetric carbons on various substituents, including enantiomeric forms (which may exist even in the absence of asymmetric carbons), rotameric forms, atropisomers, and diastereomeric forms, are contemplated within the scope of this invention. Individual stereoisomers of the compounds of the invention may, for example, be substantially free of other isomers, or may be admixed, for example, as racemates or with all other, or other selected, stereoisomers.

The inventor's studies have shown that compounds of the present invention are cytotoxic anti-cancer agents. Accordingly, the present invention also provides a method for the treatment of cancer in an animal, the method including the step of administering to the animal in need of such treatment an effective amount of a compound having the formula (1), or a pharmaceutically acceptable derivative, salt, racemate, isomer or tautomer thereof:



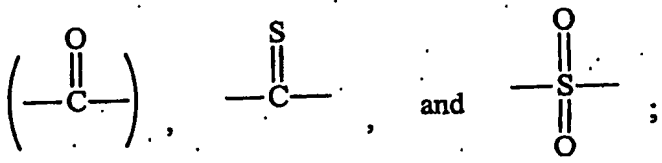
(1)

R_1 is a linking moiety;

M is a zinc binding moiety containing at least one heteroatom;

R_6 is selected from the group consisting of H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl and a nitrogen protecting group,

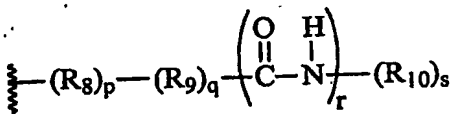
X is selected from the group consisting of



;

Y is selected from the group consisting: of $-NR_4R_5$, $-OR_4$, $-SR_4$, $-CH_2R_4$, CHR_4R_5, $C(R_4)_2R_5$, PHR_4 and PR_4R_5,

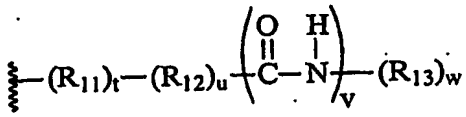
wherein R_4 is a group of formula:



wherein R_8, R_9 and R_{10} are each independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, and optionally substituted heterocycloalkyl;

p, q, r and s are each independently 0 or 1, provided that at least one of p, q or s is 1;

R_5 is H or a group of formula:



wherein R_{11}, R_{12} and R_{113} are each independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, or optionally substituted heterocycloalkyl;

t, u, v and w are each independently 0 or 1, provided that at least one of t, u and w is 1;

R_7 is a group of formula:

$$(R_{16})_z\text{-}(R_{15})_y\text{-}(R_{14})_x—$$

wherein R_{14}, R_{15} and R_{16} are independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl and optionally substituted heterocycloalkyl,

x, y and z are independently 0 and 1 with the proviso that at least one of x, y and z is 1.

In a preferred embodiment of the method of the invention the animal is a human. The compound of the invention may be administered in any suitable form well known in the art including oral administration in the form of a liquid, syrup, tablet or the like, by injection or by intravenous infusion. It is preferred that the compound is administered by intravenous infusion.

The present invention also provides pharmaceutical and/or veterinary compositions containing one or more of the compounds of the invention and a pharmaceutically acceptable, carrier, diluent or excipient. These compositions may be used in the methods of treatment discussed previously.

In a further aspect the invention provides the use of the compounds of the invention as hereinbefore described for the preparation of a medicament for the treatment of cancer.

Description of the Figures

Figure 1. Acetylation of Histones. MM96L cells were treated with 5 µg/mL of test compounds for 8 hours, before harvest and analysis of histone H4 acetylation by Triton-acetic acid-urea gel (Saito *et al.*, 1991; Qiu *et al.*, 1999). Lane 1: untreated. Lane 2: compound of example 13 Lane 3: compound of example 31. Lane 4: TSA. Non-acetylated (A), mono-acetylated (B), di-acetylated (C), tri-acetylated (D) and tetra-acetylated (E) histone H4 are indicated.

Figure 2. Acetylaton of histones MM96L cells were treated with 5 µg/ml of various compound for 8 hr, before harvest and analysis of histone H4 acetylation by Triton-acetic acid-urea gel as previously described (Saito *et al.*, 1991; Qiu *et al.*, 1999). Lane 1: untreated: lane 2: Compound of example 64; lane 3: Compound of example 31; lane 4: TSA. Non-acetylated (A), mono-acetylated (B), di-acetylated (C), tri-acetylated (D) and tetra-acetylated (E) histone H4 are indicated.

Figure 3. Induction of p21 expression. MM96L cells were treated with the compound of example 15 (10 µg/mL) and total RNA was isolated from cells, reverse transcribed using SuperScript II and oligo-dT primer, and cDNA amplified by PCR using primers specific for $p21^{WAF1/Cip1}$ and GAPDH. Lane 1, untreated; lane 2, 16 hours treatment; lane 3, 24 hours treatment; lane 4, RT-PCR negative control. Quantitation of $p21^{WAF1/Cip1}$ induction was performed by densitometric analysis using ImageQuaNT 4.2 software (Molecular Dynamics, Sunnyvale, CA) following normalisation to GAPDH product intensity. Expression of $p21^{WAF1/Cip1}$ was increased 2.1-fold above that of untreated cells at both the 16 and 24 hr time points.

Figure 4 Induction of p21 expression MM96L melanoma cells were treated with 2 compounds at a concentration of 10 µg/ml, and total RNA was isolated following 16 and 24 hrs, as described in Materials and Methods. Semi-quantitative RT-PCR was performed on the total RNA samples. Induction of mRNA for $p21^{WAF1/Cip1}$ was seen after 16 hrs treatment for both compound of example 15 and compound of example 58

Figure 5 Morphological Reversion After 24 hours. (a) Untreated normal melanocytes; (b) Normal melanocytes treated with compound of example 31 (10 µg/mL); (c) Untreated melanoma cells (MM96L); (d) MM96L treated with compound of example 31 (10 µg/mL).

Figure 6 Morphological Reversion After 24 hours. (a) Untreated normal melanocytes; (b) Normal melanocytes treated with compound of example 58 (10 µg/mL); (c) Untreated melanoma cells (MM96L); (d) MM96L treated with compound of example 58 (10 µg/mL).

Figure 7 Oral Bioavailability. Time dependent plasma concentration of compound of example 15 after oral (top) and intravenous (bottom) administration at 5 mg/kg to each of three Wistar rats.

Detailed Description of the Invention

The compounds of the invention have been found to possess cytotoxic effects against cancer cells and are therefore useful in methods for the treatment of cancer in animals especially humans. As used herein the term 'cancer' is a general term intended to encompass the more than 100 conditions that are characterised by uncontrolled abnormal growth of cells.

Examples of cancer types that may be able to be treated by the compounds of the present invention include bone cancers including Ewing's sarcoma, osteosarcoma, chondrosarcoma and the like, brain and CNS tumours including acoustic neuroma, neuroblastomas and other brain tumours, spinal cord tumours, breast cancers, colorectal cancers, endocrine cancers including adenocortical carcinoma, pancreatic cancer, pituitary cancer, thyroid cancer,

parathyroid cancer, thymus cancer, multiple endocrine neoplasma, gastrointestinal cancers including stomach cancer, esophageal cancer, Small intestine cancer, Liver cancer, extra hepatic bile duct cancer, gastrointestinal Carcinoid tumour, gall bladder cancer, genitourinary cancers including testicular cancer, penile cancer, prostrate cancer, gynaecological cancers including cervical cancer, ovarian cancer, vaginal cancer, uterus/endometrium cancer, vulva cancer, gestational trophoblastic cancer, fallopian tube cancer, uterine sarcoma, head and neck cancers including oral cavity cancer, lip cancer, salivary gland cancer, larynx cancer, hypopharynx cancer, orthopharynx cancer, nasal cancer, paranasla cancer, nasopharynx caner, leukemias including childhood leukemia, acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, hairy cell leukemia, acute promyelocytic leukemia, plasma cell leukemia, myelomas, haematological disorders including myelodysplastic syndromes, myeloproliferative disorders, aplastic anemia, Fanconi anemia, Waldenstroms Macroglobulinemia, lung cancers including small cell lung cancer, non-small cell lung cancer, lymphomas including Hodgkinsons disease, non-Hodgkinsons's lymphoma, AIDS related Lymphoma, eye cancers including retinoblastoma, intraocular melanoma, skin cancers including melanoma, non-melanoma skin cancer, merkel cell cancer, soft tissue sarcomas such as childhood soft tissue sarcoma, adult soft tissue sarcoma, Kaposi's sarcoma, urinary system cancers including kidney cancer, Wilms tumour, bladder cancer, urethral cancer, and transitional cell cancer.

Preferred cancers that may be treated by the compounds of the present invention are melanomas, skin, breast, prostrate and ovarian cancers.

Various terms used throughout the specification have meanings that will be well understood by a skilled addressee in the area. Nevertheless, for ease of reference, some of these terms will now be defined.

The term "animal" as used throughout the specification is to be understood to mean ordinarily a mammal such as a human, sheep, horse, cattle, pig, dog, cats, rat and mouse. For example, the animal may be a human subject suffering the effects of cancer.

The term "alkyl" or "alk" as employed herein alone or as part of another group refers to a monovalent saturated hydrocarbon derived radical having the number of carbons specified or if no number is specified up to 30 carbons. The term includes straight or branched saturated hydrocarbon groups. The group preferably contains from 1 to 20 carbons, more preferably from 1 to 10 carbons, even more preferably 1 to 8 carbons in the normal chain. Examples of alkyl include but are not limited to methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, isobutyl, pentyl, hexyl, isohexyl, heptyl, 4,4-dimethylpentyl, octyl, 2,2,4-trimethylpentyl, nonyl, decyl, undecyl, dodecyl, and the various branched chain isomers thereof.

The term "alkene" or "alkenyl" as used herein alone or as part of another group refers to straight or branched unsaturated monovalent hydrocarbon radical containing at least one carbon to carbon double bond. The group preferably contains from 2 to 20 carbons, preferably 2 to 12 carbons, most preferably 2 to 8 carbons in the normal chain. The group may include any number of double bonds in the normal chain and the orientation about each double bond is independently E or Z. Examples of alkenyl include but are not limited to ethenyl (vinyl), 2-propenyl, 2-butenyl, 3-butenyl, 3-pentenyl, 4-pentenyl, 2-hexenyl, 3-hexenyl, 2-heptenyl, 3-heptenyl, 3-octenyl, 3-nonenyl, 4-decenyl, 3-undecenyl, 4-dodecenyl, 4,8,12-tetradecatrienyl, and the like.

The term "alkyne" or "alkynyl" as used herein alone or as part of another group refers to a refers to straight, branched or cyclic unsaturated monovalent hydrocarbon radical containing at least one carbon to carbon triple bond in the normal chain. The group preferably contains from 2 to 20 carbons, preferably 2 to 12 carbons and more preferably 2 to 8 carbons in the normal chain. Examples of alkynyl include but are not limited to ethynyl, 2-propynyl, 3-buyynyl, 2-butynyl, 3-pentynyl, 4-pentynyl, 2-hexynyl, 3-hexynyl, 2-heptynyl, 3-heptynyl, 4-heptynyl, 2-octynyl, 3-octynyl, 4-octynyl; and the like.

The term "aryl" either alone or part of another group refers to monocyclic, bicyclic, tricyclic or polycyclic aromatic groups preferably containing from 6 to 20

carbons, more preferably from 6 to 14 carbons, even more preferably from 6 to 10 carbons. Examples of aryl include but are not limited to phenyl, 1-naphthyl, 2- naphthyl, anthracyl, phenanthryl, and benzonaphthenyl. These groups may optionally include one to three additional carbocyclic rings fused to the aromatic ring system

The term "cycloalkyl" alone or as part of another group indicates a saturated or partially unsaturated cyclic hydrocarbon preferably containing from 1 to 3 rings, including monocyclic alkyl, bicyclic alkyl (bicycloalkyl) and tricyclic alkyl (tricycloalkyl), and preferably containing a total of from 3 to 20 carbons forming the ring, preferably 3 to 10 carbons, forming the ring and which may be fused to 1 to 2 aromatic rings. Examples of cycloalkyl include but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl, adamantyl, and norbornyl

The term "heteroaryl" either alone or part of another group refers to groups containing an aromatic ring (preferably a 5 or 6 membered aromatic ring) having 1 or more heteroatoms as ring atoms in the aromatic ring with the remainder of the ring atoms being carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen. Examples of heteroaryl include thiophene, benzothiophene, benzofuran, benzimidazole, benzoxazole, benzothiazole, benzisothiazole, naphtho[2,3-b]thiophene, furan, isoindolizine, xantholene, phenoxatine, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indole, isoindole, 1H-indazole, purine, 4H-quinolidine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, carbazole, .beta.-carboline, phenanthridine, acridine, phenazine, thiazole, isothiazole, phenothiazine, oxazole, isoxazole, furazane, phenoxazine, 2-, 3-, or 4-pyridyl, 2-, 3-, 4-, 5-, or 8-quinolyl, 1-, 3-, 4-, or 5-isoquinolyl, 1-, 2-, or 3-indolyl, 2-benzothiazolyl, 2-benzo[b]thienyl, benzo[b]furanyl, 2- or 3-thienyl, or the like. More preferred examples include 2- or 3-thienyl, 2-, 3-, or 4-pyridyl, 2- or 3-quinolyl, 1-isoquinolyl, 1- or 2-indolyl, 2-benzothiazolyl, and the like. For ease of reference in the drawings heteroaryl is sometimes depicted with the following symbol.

This symbol is intended to be a shorthand notation for all heteroaryl groups whether monocyclic, bicyclic or polycyclic notwithstanding that a single ring is depicted in the shorthand notation.

The term "heterocycloalkyl " as used alone or as part of another group refers to a saturated or partially unsaturated ring, preferably containing 5, 6, 7 or 8 ring atoms which includes at least one of nitrogen, sulfur or oxygen as a ring atom and which may further be fused to one or more aromatic or non-aromatic rings. Examples of heterocycloalkyl include 2- pyrolline, 3-pyrolline, pyrollidine, 1,3 dioxolane, 2-imidazoline, 2-pyrazoline, pyrazolidine. piperidine, morpholine. 1,4-dioxane, thiomorpholine, piperazine and indoline.

The term "acyl" as used throughout the specification is to be understood to mean the groups alkyl-C(O)-, substituted alkyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, aryl-C(O)-, heteroaryl-C(O)- and heterocycloalkyl-C(O)-.

The term "alkoxy" as used throughout the specification is to be understood to mean the group "alkyl-O-". Preferred alkoxy groups include, by way of example, methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, 1,2-dimethylbutoxy, and the like.

The term "amino" as used throughout the specification is to be understood to mean a nitrogen optionally mono-, di- or tri-substituted.

The terms "halo" or "halogen" as used throughout the specification is to be understood to mean fluoro, chloro, bromo or iodo.

The term "optionally substituted" as used throughout the specification denotes that the group may or may not be further substituted or fused (so as to form a condensed polycyclic system), with one or more substituent groups. Preferably the substituent groups are one or more groups selected from alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, haloheteroaryl, halocycloalkyl, haloheterocycloalkyl, hydroxy, alkoxy, alkenyloxy, aryloxy, heteroaryloxy, cycloalkyloxy, heterocycloalkyloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, halohetoraryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheteroaryl, nitroheterocyclyoalkyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, heteroarylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl,heteroarylacyl, acylamino, diacylamino, acyloxy, alklysulphonlyoxy, arylsulphonyloxy, heterocycloalkylamino, alkylsulphonyl, arylsulphonyl, carboalkoxy, carboaryloxy, alkylthio, benzylthio, acylthio, cyano, nitro, sulfate and phosphate;

The term "Protecting group" refers to a chemical group that exhibits the following characteristics: 1) reacts selectively with the desired functionality in good yield to give a protected substrate that is stable to the projected reactions for which protection is desired; 2) is selectively removable from the protected substrate to yield the desired functionality; and 3) is removable in good yield by reagents compatible with the other functional group(s) present or generated in such projected reactions. Examples of suitable protecting groups can be found in Greene et al. (1991) Protective Groups in *Organic* Synthesis, 2nd Ed. (John Wiley & Sons, Inc., New York). Preferred amino protecting groups include, but are not limited to, benzyloxycarbonyl (CBz), t-butyloxycarbonyl (Boc), t-butyldimethylsilyl (TBDIMS), 9-fluorenylmethyloxycarbonyl (Fmoc), or suitable photolabile protecting groups such as 6-nitroveratryloxy carbonyl (Nvoc), nitropiperonyl, pyrenylmethoxycarbonyl, nitrobenzyl, dimethyl dimethoxybenzil, 5-bromo-7-nitroindolinyl, and the like. Preferred hydroxyl protecting groups include Fmoc, benzyl, t-butyl, allyl, TBDIMS, photolabile protecting groups (such as nitroveratryl oxymethyl ether (Nvom)), Mom (methoxy methyl ether), and Mem (methoxy ethoxy methyl ether). Particularly preferred protecting groups

include NPEOC (4-nitrophenethyloxycarbonyl) and NPEOM (4-nitrophenethyloxymethyloxycarbonyl).

As used throughout the specification the preferred number of carbon atoms will be represented by, for example, the phrase "C_x-C_y alkyl" which refers to an alkyl group as hereinbefore defined containing the specified number of carbon atoms. Similar terminology will apply for other variable.

Pharmaceutically acceptable derivatives and solvates of the compounds of the invention are also contemplated herein. The term "pharmaceutically acceptable derivative" as used throughout the specification is to be understood to mean a compound that is a drug precursor, which, upon administration to a subject, undergoes chemical conversion by metabolic or chemical processes to yield a compound of formula (1) or a salt and/or solvate thereof. The term is used interchangeably with the term 'prodrug'.

The term "solvate" as used throughout the specification is to be understood to mean a physical association of a compound of this invention with one or more solvent molecules. This physical association involves varying degrees of ionic and covalent bonding, including hydrogen bonding. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. "Solvate" encompasses both solution-phase and isolatable solvates. Non-limiting examples of suitable solvates include ethanolates, methanolates, and the like. "Hydrate" is a solvate wherein the solvent molecule is H_2O.

The term "composition" as used throughout the specification is to be understood to mean a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

The term "therapeutically effective amount" or "therapeutic amount" is an amount sufficient to effect beneficial or desired clinical results. An effective amount can be administered in one or more administrations. An effective

amount is typically sufficient to palliate, ameliorate, stabilize, reverse, slow or delay the progression of the disease state.

Compound Design

The compounds were designed on the basis that human histone deacetylases (HDACs) are known to regulate the equilibrium between acetylated and deacetylated nuclear proteins known as histones, and that this control in turn influences the degree of interaction between histones and the DNA in which histones are normally wrapped. One role for histone deacetylases then is to increase the proportion of histones wrapped in DNA, and inhibitors of this enzyme can thus enhance the unwrapping.

The specific molecular interactons between DNA and histones are mediated through lysine side chains of histones. Histone lysines possess side chains consisting of a $-(CH_2)_4-NH_2$ terminus which when acetylated ($-(CH_2)_4-NHCOCH_3$), inserts into the active site of HDAC enzymes and makes contact with a zinc ion.

The three dimensional structure of a bacterial HDAC enzyme analogue (HDLP) has been solved both as the native enzyme, and co-crystallized with the HDAC inhibitors trichostatin A and suberoylanilide hydroxamic acid (SAHA). HDLP shares ~32% homology with HDAC1 and deactetylates histones *in vitro*. High sequence homology is observed within the hydrophobic tubular catalytic active site, ~11 Å deep but narrowing to ~4 Å at the active site and terminating at a divalent zinc cation, activated water molecule, and histidine-aspartate charge-relay system. Most of the residues in the HDLP structure that interact directly with trichlorstatin are highly conserved among all the HDACs, but there is less conservation in adjoining residues, most notably on the enzyme surface which has a number of shallow pockets surrounding the active site channel.

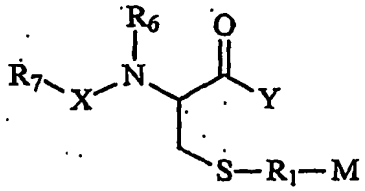
Docking of trapoxin B into the HDLP crystal structure using a combination of conformational searching (MACROMODEL) and a genetic docking algorithm (GOLD) identified tight binding conformations in which the aliphatic side chain had inserted into the tubular pocket of the active site, with the Phe side chains

in contact with the shallow pockets of the enzyme surface. These aromatic groups represent important foliage on the cyclic tetrapeptide scaffold for tight enzyme binding, and similar groups are represented in related naturally occurring cyclic tetrapeptides (Phe, Trp, Tyr). However, cyclic tetrapeptides offer limited scope for potential therapeutics due to their difficulty of synthesis, problematic stability, and conformational homogeneity. It was generally conceived that active compounds could be developed by mimicking the key enzyme binding regions of Trapoxin B, which would include a zinc chelator tethered to a branched capping group capable of reproducing the approximate positions and orientations of the Phe side chains, on a much simplified template. It was envisaged that a tripeptide incorporating similar surface binding groups to those found in the potent naturally occurring cyclic tetrapeptide inhibitors (hydrophobic, aromatic, basic) would be able to span the surface binding domain of Trapoxin B, while a hydrophobic tether terminating at a hydroxamic acid would ensure firm zinc binding in the catalytic core.

Analysis of the problem led to the conclusion that cysteine like frameworks have the potential to meet the above requirements as they provide the appropriate functionality and orientation to mimic the cyclic peptide. In particular L-Cysteine (amino acid side chain being $-(CH_2)$-SH) was selected as the ideal core building block for these hybrid inhibitors, as it provides a convenient source of asymmetry and can be appended to in three directions. The longer carbon-sulfur bond lengths, as compared to carbon-carbon bonds, provide a tether of intermediate length between the five to six methylene units thought to be optimal from studies of analogues of suberoylanilide hydroxamic acid (SBHA) and azelaic bishydroxamic acid (ABHA) both known inhibitors of HDACs. Additionally the high polarization of sulfur improves water solubility, but does not compromise unacceptably the hydrophobic nature of the aliphatic tether

Synthetic studies in this area were therefore directed towards the use of cysteine like frameworks as a building block from which improved compounds could be developed. These studies led to the development of the compounds of the invention.

The present invention provides a compound having the formula (1), or a pharmaceutically acceptable derivative, salt, racemate, isomer or tautomer thereof:



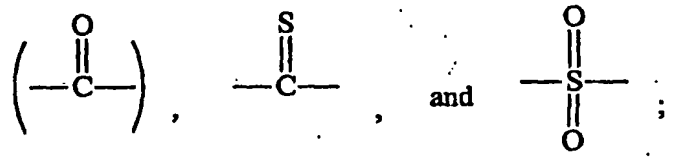
(1)

R_1 is a linking moiety;

M is a zinc binding moiety containing at least one heteroatom;

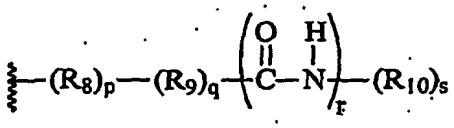
R_6 is selected from the group consisting of H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl and a nitrogen protecting group,

X is selected from the group consisting of



Y is selected from the group consisting of: $-NR_4R_5$, $-OR_4$, $-SR_4$, $-CH_2R_4$, $-CHR_4R_5$, $-C(R_4)_2R_5$, PHR_4 and PR_4R_5,

wherein R_4 is a group of formula:

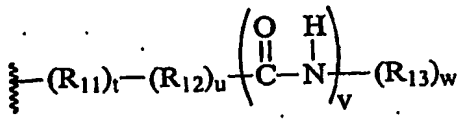


wherein R_8, R_9 and R_{10} are each independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally

substituted aryl, optionally substituted heteroaryl, and optionally substituted heterocycloalkyl;

p, q, r and s are each independently 0 or 1, provided that at least one of p, q or s is 1;

R_5 is H or a group of formula:



wherein R_{11}, R_{12} and R_{113} are each independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, or optionally substituted heterocycloalkyl;

t, u, v and w are each independently 0 or 1, provided that at least one of t, u and w is 1;

R_7 is a group of formula:

$$(R_{16})_z\text{-}(R_{15})_y\text{-}(R_{14})_x\text{—}$$

wherein R_{14}, R_{15} and R_{16} are independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl and optionally substituted heterocycloalkyl,

x, y and z are independently 0 and 1 with the proviso that at least one of x, y and z is 1.

As would be clear to a skilled addressee any number of suitable moieties can be used as the linking moiety of the compounds of the invention. It is typical, however, that the linking moiety is a hydrocarbyl moiety that is unbranched. Moieties of this type are the simplest to produce and are found to not interfere with the activity of the remainder of the compound. It is preferred that the linker has between 1-9 atoms in the normal chain, preferably between 1 and 4 atoms in the normal chain.

In addition the zinc binding moiety can be chosen so that it is any suitable moiety that will bind to zinc. There are a number of suitable zinc binding moieties well known in the art. Examples of well known zinc binding moieties include sulfur donors (such as HS-R, wherein R is defined above), amine containing compounds (primary, secondary, tertiary amines), heterocyclic amines, carboxylates, amino acids, thiolates, dithiocarbamates, phosphorodidithiolates and the like. Some examples of suitable moieties within these subsets are as follows:

Sulfur donors (thioproline, penicillamine, cysteine, 2-mercaptoethylamine, glutathione, methionine, thiosulfate, N-acetylcysteine, penicillaminedisulfide, thiomalate, and 2,3-dimercaptosuccinate

Aliphatic amines (histamine, trien, Me4en)

Heterocyclic amines (pipicolate, nicotinate, picolinate, 8-hydroxyquinoline, bicinchoninate, bipy, phendisulfonate)

Carboxylates (acetate, propionate, tartrate, succinate, malate, gluconate, betahydroxybutyrate, lactate, salicylate, citrate, ascorbate, oxalate, EDTA)

Amino acids (gly, arg, asn, glu, asp, glygly, glyglygly, glyglyhis, pro, 2,3-diaminopropionate, 2-amino-2-deoxygluconate, his)

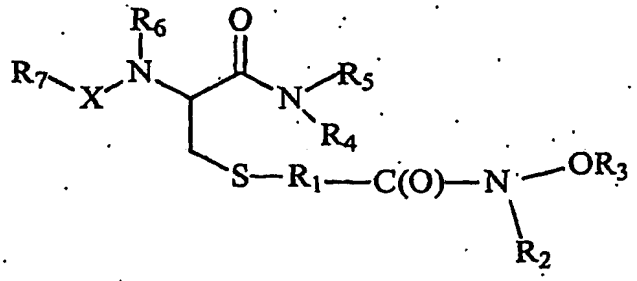
It is preferred that the zinc binding ligand is a hydroxamic acid derivative.

As with all chemical families there are a number of preferred embodiments within the scope of the general formula.

It is preferred, for example that the group Y is a group of formula $-NR_4R_5$.

It is particularly preferred that the zinc binding moiety containing a heteroatom is a hydroxamic acid derivative, preferably a group of formula $-C(O)-NR_2-OR_3$ where R_2 is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, or a nitrogen protecting group and R_3 is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl or an oxygen protecting group;

Accordingly in a preferred embodiment the present invention provides a compound having the formula (2), or a pharmaceutically acceptable derivative, salt, racemate, isomer or tautomer thereof:



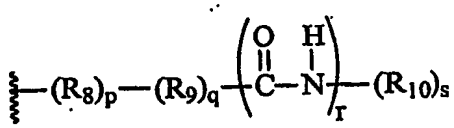
(2)

R_1 is optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 alkenyl or optionally substituted C_1-C_4 alkynyl;

R_2 is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, or a nitrogen protecting group;

R_3 is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl or an oxygen protecting group;

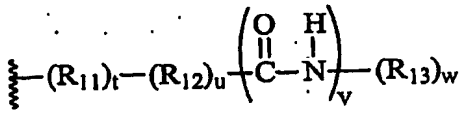
R_4 is a group of formula:



wherein R_8, R_9 and R_{10} are each independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, and optionally substituted heterocycloalkyl;

p, q, r and s are each independently 0 or 1, provided that at least one of p, q or s is 1;

R_5 is H or a group of formula:

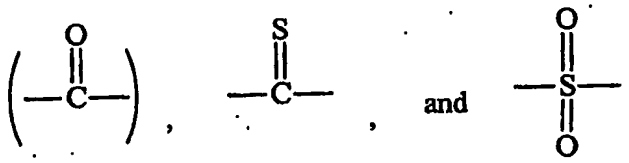


wherein R_{11}, R_{12} and R_{113} are each independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, or optionally substituted heterocycloalkyl;

t, u, v and w are each independently 0 or 1, provided that at least one of t, u and w is 1.

R_6 is selected from the group consisting of H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl and a nitrogen protecting group,

X is selected from the group consisting of



R_7 is a group of formula:

$$(R_{16})_z\text{-}(R_{15})_y\text{-}(R_{14})_x—$$

wherein R_{14}, R_{15} and R_{16} are independently selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl and optionally substituted heterocycloalkyl,

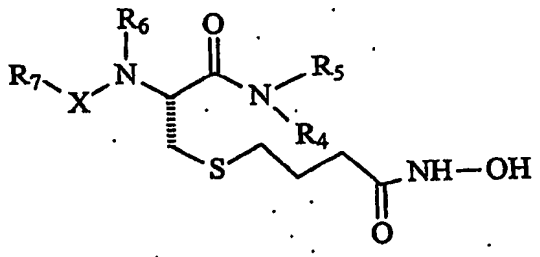
x, y and z are independently 0 and 1 with the proviso that at least one of x, y and z is 1.

As stated previously there are a number of compounds within the scope of the general structural formula that are preferred. There are therefore a number of preferred variables for each of the substituents in the general formula. For example it is preferred that R_1 is optionally substituted C_1-C_4 alkyl, more preferably optionally substituted C_2-C_3 alkyl, even more preferably optionally substituted C_3 alkyl, most preferably propyl.

It is preferred that R_2 is either H, optionally substituted C_1-C_4 alkyl or a nitrogen protecting group, more preferably H or a nitrogen protecting group, most preferably H.

It is preferred that R_3 is either H, optionally substituted C_1-C_4 alkyl or an oxygen protecting group, more preferably H or an oxygen protecting group, most preferably H.

In a most preferred embodiment, the compounds are of formula (2a).



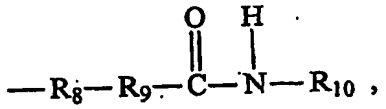
(2a)

In the compounds of the invention it is particularly preferred that X is a carbonyl group.

It is preferred that R_5 is H.

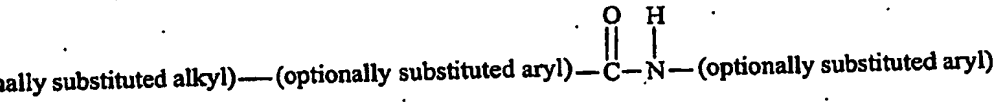
It is preferred that R_6 is either H or a nitrogen protecting group, most preferably H.

In one preferred embodiment the group R_4 is of the formula

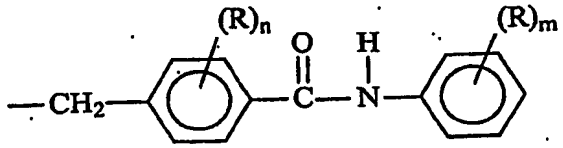


wherein R_8, R_9 and R_{10} are as defined above.

In this embodiment it is particularly preferred that R_4 is of the formula:



In the most preferred form of this embodiment R_4 is a group of the formula.



wherein each R is independently selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, haloheteroaryl, halocycloalkyl, haloheterocycloalkyl, hydroxy, alkoxy, alkenyloxy, aryloxy, heteroaryloxy, cycloalkyloxy, heterocycloalkyloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, halohetoraryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheteroaryl, nitroheterocyclyoalkyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, heteroarylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl,heteroarylacyl, acylamino, diacylamino, acyloxy, alklysulphonlyoxy, arylsulphonyloxy, heterocycloalkylamino, alkylsulphonyl, arylsulphonyl, carboalkoxy, carboaryloxy, alkylthio, benzylthio, acylthio, cyano, nitro, sulfate and phosphate;

n is 0-4, and

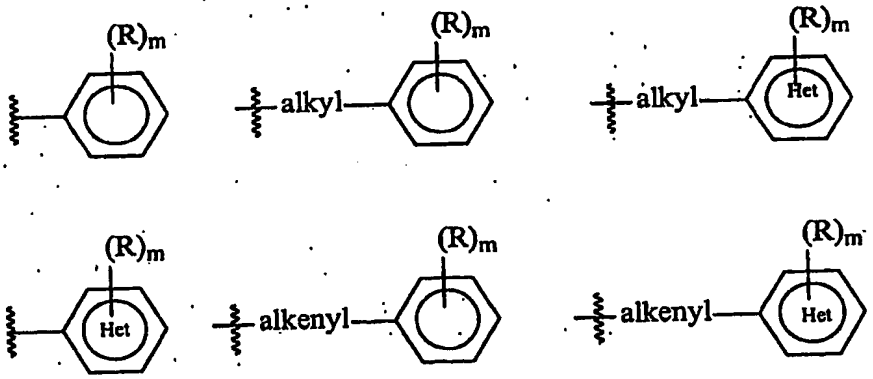
m is 0-5.

In an another preferred embodiment of the invention R_4 is selected from the group consisting of: optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted cycloalkyl, optionally substituted heteroaryl, optionally substituted heterocycloalkyl, optionally substituted arylalkyl, optionally substituted heteroarylalkyl, optionally substituted cycloalkylalkyl, optionally substituted heterocycloalkylalkyl, optionally substituted aryl alkenyl, optionally substituted heteroaryl alkenyl, optionally substituted cycloalkyl alkenyl, optionally substituted heterocycloalkyl alkenyl, optionally substituted aryl alkynyl; optionally substituted heteroaryl alkynyl optionally substituted cycloalkyl alkynyl, optionally substituted heterocycloalkyl alkynyl.

In this embodiment it is particularly preferred that R_4 is selected from optionally substituted aryl, optionally substituted cycloalkyl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted arylalkyl, optionally

substituted heteroarylalkyl, optionally substituted cycloalkyl alkyl, optionally substituted alkyl aryl, optionally substituted alkyl heteroaryl, optionally substituted alkyl heterocycloalkyl.

In a most preferred embodiment of the invention R_4 has one of the following Formulas.



Wherein each R is independently selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, haloheteroaryl, halocycloalkyl, haloheterocycloalkyl, hydroxy, alkoxy, alkenyloxy, aryloxy, heteroaryloxy, cycloalkyloxy, heterocycloalkyloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, halohetoraryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheteroaryl, nitroheterocyclyoalkyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, heteroarylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl,heteroarylacyl, acylamino, diacylamino, acyloxy, alklysulphonlyoxy, arylsulphonyloxy, heterocycloalkylamino, alkylsulphonyl, arylsulphonyl, carboalkoxy, carboaryloxy, alkylthio, benzylthio, acylthio, cyano, nitro, sulfate and phosphate;

and each m is from 0-5.

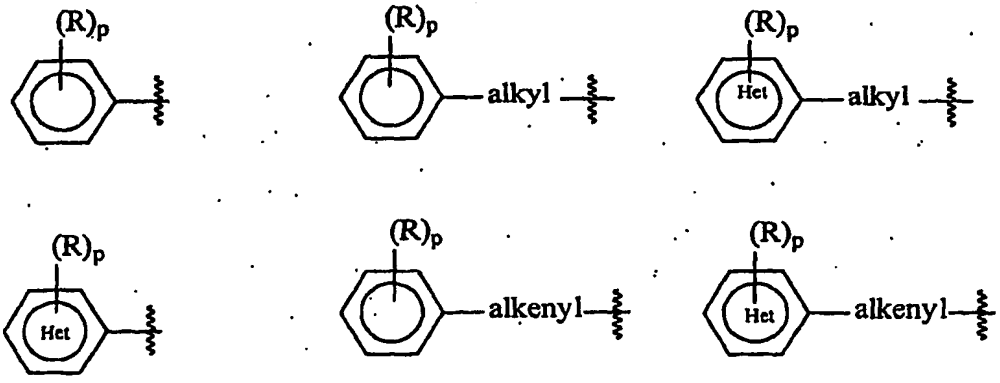
Preferred values of R as substituents on R_4 are dialkyl amino, acyl, aryl, carboalkoxy, benzyl, cycloalkyl, heteroaryl, hydroxy, halo and cyano.

Particularly preferred values of R_4 are dimethyl amino, diethyl amino, bromo, phenyl and benzyl.

In the compounds of the invention it is preferred that R_7 is selected from the group consisting of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted cycloalkyl, optionally substituted heteroaryl, optionally substituted heterocycloalkyl, optionally substituted aryl alkyl, optionally substituted heteroaryl alkyl, optionally substituted cycloalkyl alkyl, optionally substituted heterocycloalkyl alkyl, optionally substituted aryl alkenyl, optionally substituted hetero alkenyl, optionally substituted cycloalkyl alkenyl, optionally substituted heterocycloalkyl alkenyl, optionally substituted aryl alkynyl, optionally substituted heteroaryl alkynyl, optionally substituted cycloalkyl alkynyl, optionally substituted and heterocycloalkyl alkynyl.

It is even more preferred that R_7 is optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl alkyl, optionally substituted alkenyl, optionally substituted aryl alkenyl.

It is most preferred that R_7 has one of the following formula:



Wherein each R is independently related from the group consisting of alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, haloheteroaryl, halocycloalkyl,

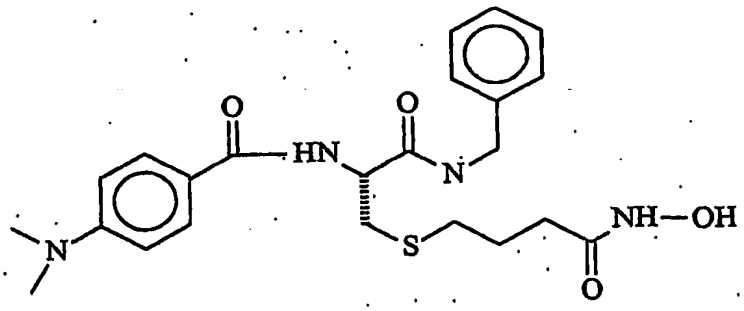
haloheterocycloalkyl, hydroxy, alkoxy, alkenyloxy, aryloxy, heteroaryloxy, cycloalkyloxy, heterocycloalkyloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, halohetoraryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheteroaryl, nitroheterocyclyoalkyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, heteroarylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl,heteroarylacyl, acylamino, diacylamino, acyloxy, alklysulphonlyoxy, arylsulphonyloxy, heterocycloalkylamino, alkylsulphonyl, arylsulphonyl, carboalkoxy, carboaryloxy, alkylthio, benzylthio, acylthio, cyano, nitro, sulfate and phosphate;

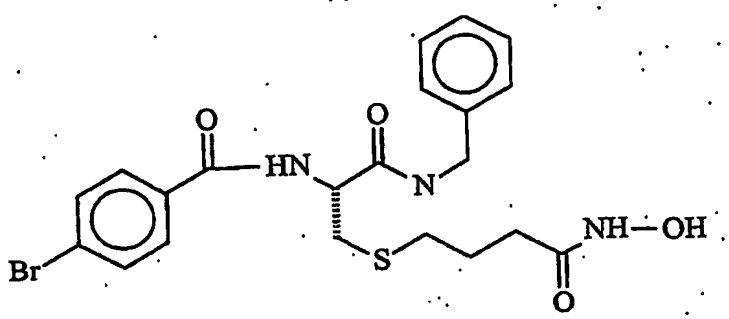
and each p is from 0-5.

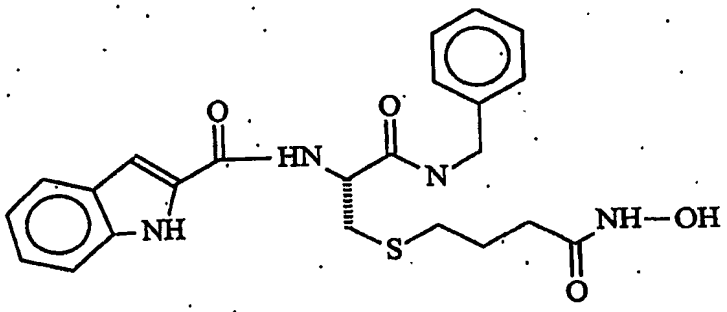
Particularly preferred values of R as a substituent on an R_7 group are dialkylamino, alkoxy, halo, aryl, alkyl, hydroxy, nitro and arylamino.

Preferred compounds of the invention include those listed in tables 1 to 3 in the examples.

Particularly preferred compounds are as follows.







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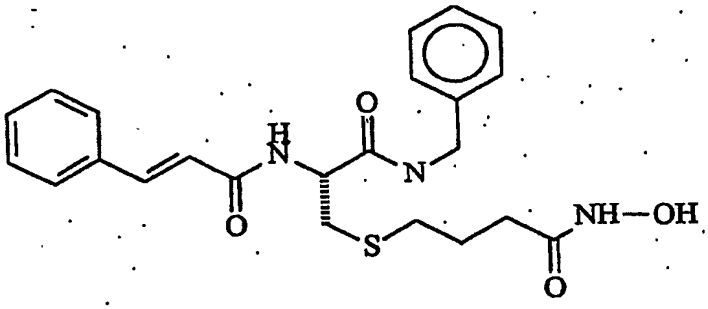

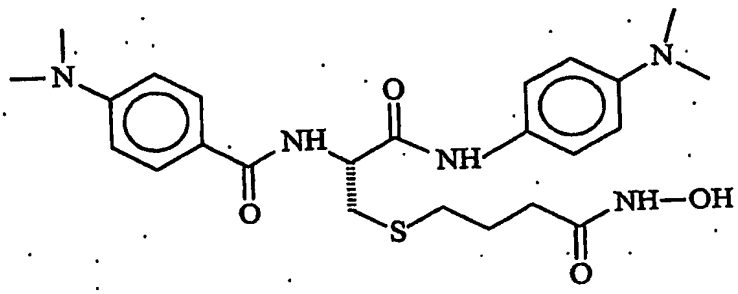

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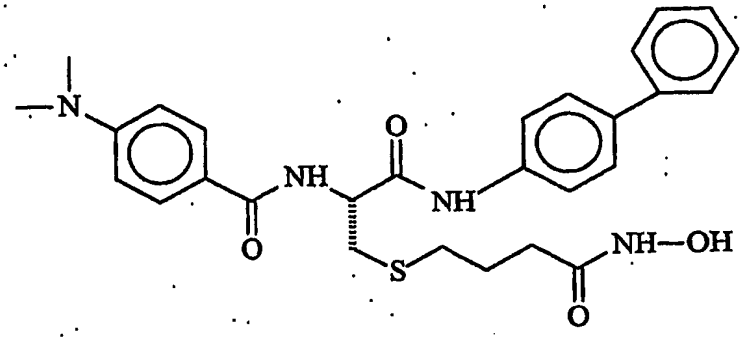
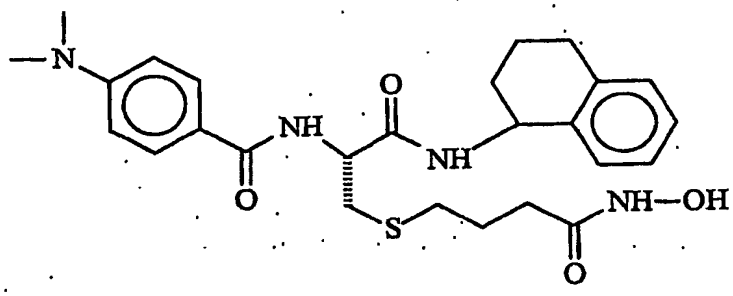
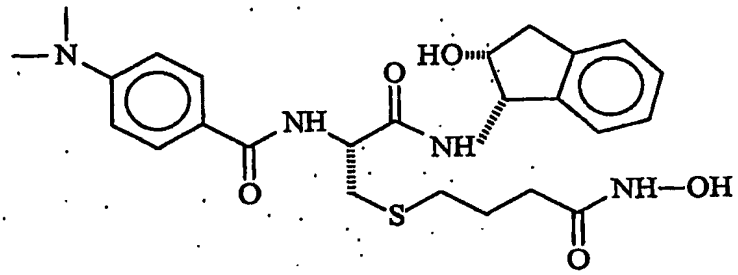
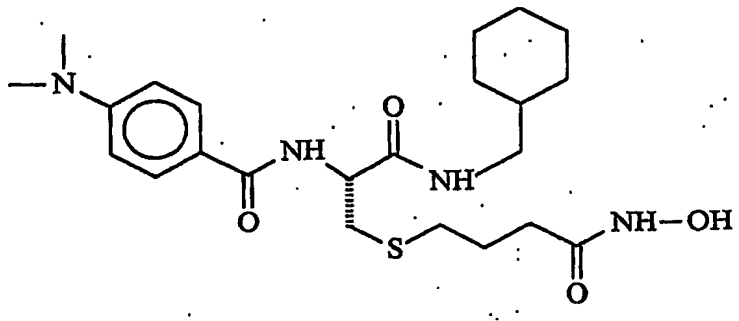


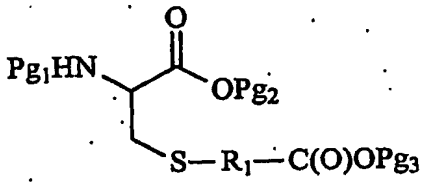






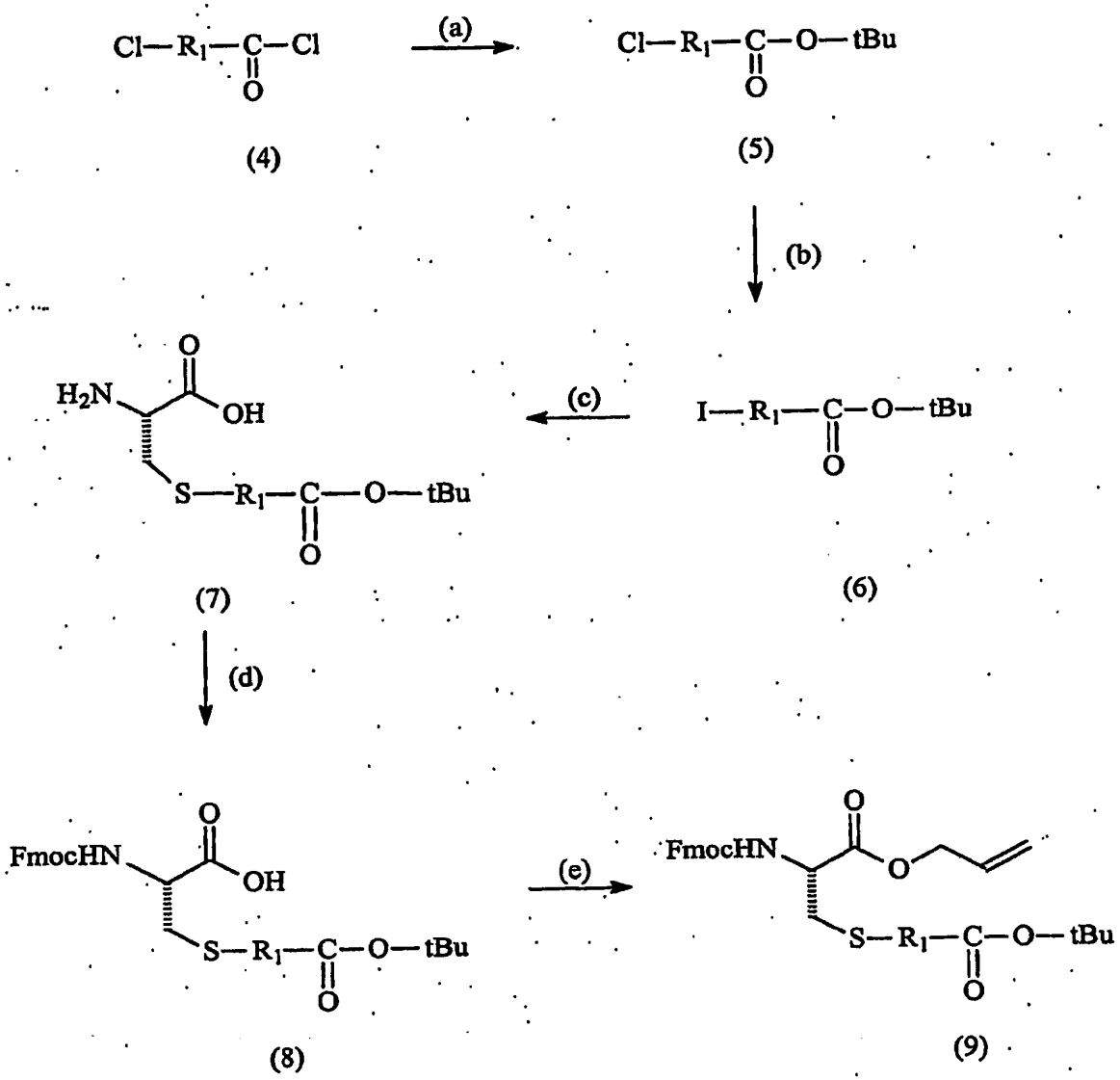
Synthesis of the compounds of the invention.

The compounds of formula (1) may be generated in a number of ways depending on the synthetic strategy adopted and the available starting materials: As would be clear to a skilled addressee the exact method utilised will depend on the available starting materials. In general however the applicants have identified that an efficient methodology for producing the compounds of the invention is to progress through an advanced intermediate of the formula (3):



(3)

Wherein Pg_1 is a protecting group for nitrogen and Pg_2 and Pg_3 are protecting groups for oxygen and R_1 is as previously defined. The protecting groups in formula (3) may be any suitable groups that are suitably adapted for the remaining steps of the process. It is important, however that the two carboxylic acid protecting groups can be differentially de-protected so that the two groups can be separately functionalised. A preferred form of the compound of formula (3) can be made utilising the reaction sequence outlined in scheme 1. Modifications to this general scheme can be made to produce compounds of formula (3) with other protecting groups and/or general structures. The extent of the modifications and the way in which could be done are well within the ambit of a skilled addressee in the art.



(a) ^{t}BuOH, Pyridine (b) NaI, THF (c) Cys, NaOH, MeOH (d) Fmoc-OSu; NaHCO3, THF, Water (e) allyl bromide, DMF, K_2CO_3

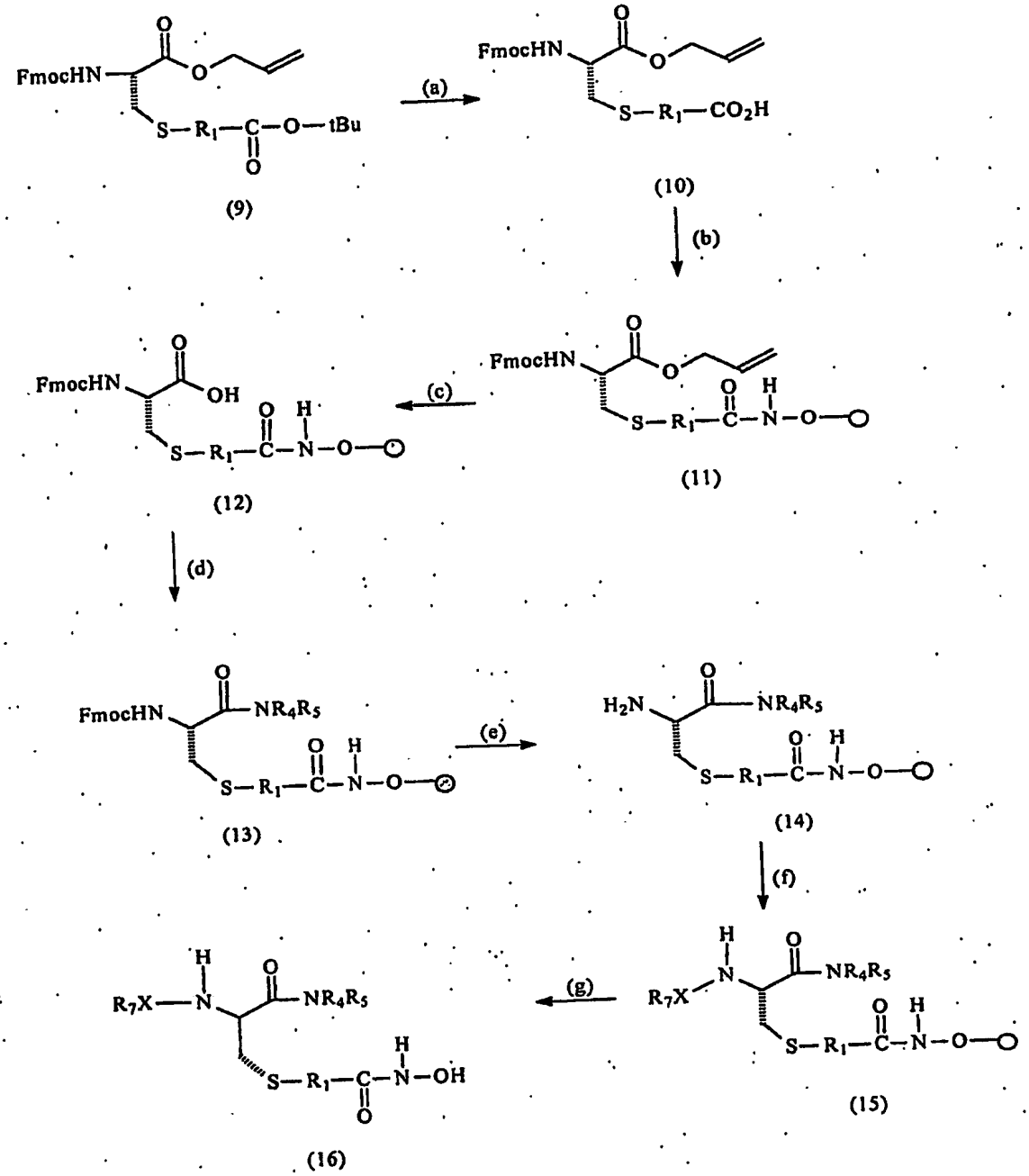
Scheme 1

Referring to scheme 1 carboxylic acid chloride (4) with the desired R_1 group is converted to the protected form by reaction with tertiary butanol in pyridine to produce the t-butyl protected form (5). The choice of protecting group will vary depending on a number of factors including the identity of the further protecting group chosen. The choice of a suitable protecting group will typically not cause

difficulty for a skilled addressee and can vary greatly with the preferred group being t-butyl..

The protected carboxylic acid (5) is then reacted with sodium iodide to produce the iodinated derivative (6). This is then reacted with an appropriate thio derivative such as cysteine to produce intermediate (7). This compound is then protected at both the C and N termini. Accordingly it is preferred that the compound is reacted with a nitrogen protecting group such as Fmoc to produce the N-protected compound (8) which is then in turn reacted with allyl bromide to produce the final differentially protected compound (9). In the preferred embodiment of the invention R_1 is propyl and the production of the preferred compounds follows an analogous procedure as that shown in scheme 1 with the starting compound (4) being the acid chloride of 4-chlorobutyric acid. In order to vary the group R_1 in the final compounds of the invention all that is required is that the starting material (4) contain the suitable R_1. In general a skilled worker in the field will easily be able to produce a wide range of compounds of general formula (4) with different values of R_1 from commercially available starting materials. In addition whilst in the reaction scheme shown above the iodinated compound (6) is reacted with naturally occurring cysteine it could equally be reacted with the unnatural isomer or even a mixture of isomers.

The compounds of formula (9) are then converted into the compounds of the invention utilising the general procedure given in scheme 2.



(a) TFA, (b) HATU, DIPEA, 2 Chlorotrityl resin, DMF, (c) $Pd(PPh_3)_4$, DMBA, (d) HNR_4R_5, HBTU, DIPEA, DMF, (e) Piperidine, (f) R_7X-L, HBTU, DIPEA, DMF, (g) TFA.

Scheme 2

Thus the compound of formula (9) is de-protected by reaction with TFA to differentially remove the t-butyl protecting group and form compound (10). This de-protected compound is then reacted with an appropriately modified resin to

immobilise the compound on the resin and form immobilised compound (11). The immobilised compound is then treated with palladium to remove the allyl protecting group to form immobilised acid (12). Reaction of acid (12) with an appropriately substituted nucleophillic compound such as amine of formula (HNR_4R_5) produces advanced compound (13). This is then reacted with piperidine to remove the Fmoc protecting group to produce the free amine (14). Reaction of amine (14) with a group of formula R_7XL where L is a leaving group then produces compound (15). The compound can then be removed from the solid support by reaction with TFA under appropriate conditions to form the compound (16) of the invention.

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallisation, column chromatography, thin-layer chromatography, thick-layer (preparative) chromatography, distillation, HPLC or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by reference to the examples provided herein. However, other equivalent separation or isolation procedures can also be used.

The present invention also provides a method for the treatment of cancer in an animal, the method including the step of administering to the animal in need of such treatment an effective amount of a compound having the formula (1), or a pharmaceutically acceptable derivative, salt, racemate, or isomer thereof:

The compounds of this invention may be administered in compositions such as tablets, capsules or elixirs for oral administration, suppositories, sterile solutions or suspensions for injectable administration, and the like, or incorporated into shaped articles. Typical adjuvants which may be incorporated into tablets, capsules and the like are a binder such as acacia, corn starch or gelatin, and excipient such as microcrystalline cellulose, a disintegrating agent like corn starch or alginic acid, a lubricant such as magnesium stearate, a sweetening agent such as sucrose or lactose, or a flavoring agent. When a dosage form is a capsule, in addition to the above materials it may also contain a liquid carrier

such as water, saline, fatty oil. Other materials of various types may be used as coatings or as modifiers of the physical form of the dosage unit. Sterile compositions for injection can be formulated according to conventional pharmaceutical practice. For example, dissolution or suspension of the active compound in a vehicle such as an oil or a synthetic fatty vehicle like ethyl oleate, or into a liposome may be desired. Buffers, preservatives, antioxidants and the like can be incorporated according to accepted pharmaceutical practice.

While the preferred route of administration is oral, other methods of administration are also anticipated such as intravenously (bolus and/or infusion), subcutaneously, intramuscularly, transdermally, colonically, rectally, nasally or intraperitoneally, employing a variety of dosage forms such as suppositories, implanted pellets or small cylinders, aerosols, injectable formulations and topical formulations such as ointments, drops and dermal patches. The compounds of this invention could be incorporated into shaped articles such as implants which may employ inert materials such as biodegradable polymers or synthetic silicones, for example, Silastic, silicone rubber or other polymers commercially available.

The compounds of this invention may also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of lipids, such as cholesterol, stearylamine or phosphatidylcholines.

Formulations of the compounds of this invention are prepared for storage or administration by mixing the compound having a desired degree of purity with physiologically acceptable carriers, excipients, stabilisers etc., and may be provided in sustained release or timed release formulations. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical field, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co., (A. R. Gennaro edit. 1985). Such materials are nontoxic to the recipients at the dosages and concentrations employed, and may include buffers such as phosphate, citrate, acetate and other organic acid salts, antioxidants such as ascorbic acid, low molecular weight (less than about ten

residues) peptides such as polyarginine, proteins, such as serum albumin, gelatin, or immunoglobulins, hydrophilic polymers such as polyvinalpyrrolidinone, amino acids such as glycine, glutamic acid, aspartic acid, or arginine, monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives; glucose, mannose or dextrins, chelating agents such as EDTA, sugar alcohols such as mannitol or sorbitol, counterions such as sodium and/or nonionic surfactants such as Tween, Pluronics or polyethyleneglycol.

Animals in need of treatment using the compounds of this invention can be administered dosages that will provide optimal efficacy. The dose and method of administration will vary from animal to animal and be dependent upon such factors as the type of mammal being treated, its sex, weight, diet, concurrent medication, overall clinical condition, the particular compounds employed, the specific use for which these compounds are employed, and other factors which those skilled in the medical arts will recognise.

Therapeutically effective dosages may be determined by either *in vitro* or *in vivo* methods. For each particular compound of the present invention, individual determinations may be made to determine the optimal dosage required. The range of therapeutically effective dosages will naturally be influenced by the route of administration, the therapeutic objectives, and the condition of the patient. It may be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. The determination of effective dosage levels, that is, the dosage levels necessary to achieve the desired result, will be within the knowledge of one skilled in the art. For example it is typical that for any compound used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from cell culture assays. Then, the dosage can be formulated for use in animal models so as to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal inhibition of the PK activity). Such information can then be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD_{50} and ED_{50}. Compounds that exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition- (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Typically, applications of compound are commenced at lower dosage levels, with dosage levels being increased until the desired effect is achieved.

Generally a dosage of as little as about 1-2 milligram (mg) per kilogram (kg) of body weight is suitable, but preferably as little as 1 mg/kg and up to about 100 mg/kg may be used. Preferably, a dosage from 2 mg/kg to about 40 mg/kg is used. Most preferably, the dose is between 4 mg/kg to about 8 mg/kg. Any range of doses can be used. Generally, a compound, salt thereof, prodrug thereof, or combination of the present invention can be administered on a daily basis one or more times a day, or one to four times a week, either in a single dose or separate doses during the day. Twice-weekly dosing over a period of at least several weeks is preferred, and often dosing will be continued over extended periods of time and possibly for the lifetime of the patient. However, the dosage and the dosage regimen will vary depending on the ability of the patient to sustain the desired and effective plasma levels of the compounds of the present invention, or salt or prodrug thereof, in the blood.

In practicing the methods of this invention, the compounds of this invention may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compounds of this invention may be co-administered along with other compounds typically prescribed for these conditions according to generally accepted medical practice. For example, compounds of this invention may be used in combination with DNA methyltransferase inhibitors (as described in Herman JG and Baylin SB (2003) NEJM 349, 2042-2054). Such inhibitors may include but are not limited to 5-azacytidine, deoxy-5-azacytidine, or zebularine.

The compounds of this invention may also be delivered by the use of antibodies, antibody fragments, growth factors, hormones, or other targeting moieties, to which the compound molecules are coupled. The compounds of this invention may also be coupled with suitable polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxy-propyl-methacrylamide-phenol, polyhydroxyethyl-aspartamide-phenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, the compounds of this invention may be coupled to a biodegradable polymer for achieving controlled release of a drug. Examples of such polymers include polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross linked or amphipathic block copolymers of hydrogels. Polymers and semipermeable polymer matrices may be formed into shaped articles, such as valves, stents, tubing, prostheses and the like.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilise the compounds of the present invention and practice the claimed methods.

The following abbreviations are used in the examples and elsewhere throughout the specification:

Ac = acetyl;

DCM = Dichloromethane;
DIPEA = diisopropylethylamine;
DMAP = 4-(Dimethylamino)pyridine;
DMBA = 1,3-Dimethylbarbituric acid;
DMF = dimethylformamide;
EtOAc = Ethyl acetate;
Fmoc-OSu = 9-Fluorenylmethyloxycarbonyl-N-hydroxysuccinimide;
HATU=O-(7-azabenzotriazol-1-yl)-1,1,3,3tetramethyluroniumhexafluoro phosphate;
HBTU=[(benzotriazolyl)oxy]-N',N',N',N'-tatramethyluroniumhexafluorophosphate; rpHPLC = reverse phase high performance liquid chromatography;
LRMS = Low resolution mass spectroscopy;
TFA = trifluoroacetic acid;
THF = tetrahydrofuran.

The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

Examples of Preferred Embodiments of the Invention

General methods

^{1}H NMR spectra were recorded on either a Bruker ARX 500 MHz or a Varian 300 MHz NMR spectrometer. Semi preparative scale rpHPLC separations were performed on a Phenomenex Luna 5µ C18(2) 250 x 21 mm column run at 20 mL/minute using gradient mixtures of water/0.1% TFA (A) and water (10%)/acetonitrile (90%)/0.1% TFA (B), and product fractions were always lyophilized to dryness. Preparative scale rpHPLC separations were performed on a Vydac 218TP101550 50 x 250 mm column run at 70 mL/minute using gradient mixtures of A and B. Accurate mass determinations were performed on an API QSTAR mass spectrometer using electron impact ionization. Water octanol partition coefficients (Log D) were calculated using PALLAS prolog D 2.1. Molecular modeling was performed on an SGI Octane R12000, with

minimization calculation performed with the cff91 force field using the Discover Module within Insight II.

Example 1

Coupling of acid to resin (general method)

Commercially available N-Fmoc hydroxylamine 2-chlorotrityl resin (0.77 mmol/g, 10 g, 7.7 mmol) was shaken gently with 1:1 piperidine:DMF (30 mL) over night, and then flow washed with DMF for 1 minute. In a separate flask, HATU (3.0 g, 7.8 mmol) was added to a solution of the acid (7.8 mmol) and DIPEA (5.3 mL, 31.2 mmol) dissolved in DMF (10 mL), and the resulting solution stirred gently for 5 minutes. The HATU activated acid was then added in one portion to the deprotected resin, and the resin was shaken gently for 1 hour. After washing the resin well with DMF, the resin loading was determined. The unreacted resin was then acylated by addition of a solution of acetic anhydride (842 mg, 7.8 mmol) and DIPEA (5.3 mL, 31.2 mmol) in DMF (20mL) with shaking for 2 minutes, followed by thorough washing with DMF.

Example 2

Coupling of Acid moiety with functional group to add NR_4R_5 group (general method)

The resin (0.45 mmol/g, 200mg, 0.09 mmol) was shaken in DMF (1 mL) for 10 minutes, and then DIPEA (122 µL, 0.72 mmol) and 0.5 M HBTU in DMF (360 µL, 0.18 mmol) were introduced and shaking continued for a further 5 minutes. The amine (0.25 mmol) was then added, and shaking continued for a further 1 hour.. After washing the resin well with DMF, cleavage of a small portion of resin and analysis by mass spectroscopy generally indicates 60-85% conversion to the amide.

Example 3

Coupling of amine moiety with functional group to add R_7X group (general method)

The resin was shaken in DMF (1 mL) for 10 minutes, the DMF removed, and then 1:1 piperidine:DMF (1 mL) added. After shaking for 5 minutes the piperidine:DMF was removed, and the resin washed well with DMF. This

procedure was repeated two more times. In a separate flask 0.5 M HBTU (180 µL, 90 µmol) in DMF was added to a solution of the desired acid (90 µM) and DIPEA (76 µL, 450 µmol) in DMF (1 mL), and the resulting solution stirred for 5 minutes before being added in one portion to the resin. The resin was shaken for 1 hour, and then washed well with DMF. Cleavage of a small portion of resin and analysis by mass spectroscopy generally indicates 100% conversion to the amide.

Example 4

Cleavage of immobilised compound from resin (general method)

The resin was washed well with DCM, and then drained. TFA:water (99:1, 1mL) was added, and the resin shaken for 20 minutes. The TFA was collected, and the resin washed with a further 1 mL of TFA. The TFA was removed by distillation. Purification was performed by rpHPLC, and hydroxamates confirmed to be greater than 95% pure by analytical rpHPLC and ^{1}H NMR spectroscopy.

Production of a preferred Intermediate

Example 5

4-Chloro-butyric acid tert-butyl ester.

4-Chlorobutyryl chloride (16.6 mL, 147 mmol) was added drop wise to a cooled (0 °C) solution of DMAP (10 mg) in equal portions of tert-butanol (50 mL) and pyridine (50 mL). After complete addition of the acid chloride, the resulting suspension was stirred for 1 hour, and then solvent removed under reduced pressure. The residue was dissolved in EtOAc (500 mL), and washed successively with saturated $NaHCO_3$ and NaCl solutions. The organic layer was dried over magnesium sulfate, and solvent removed to provide the tert-butyl ester as a clear oil (22.3 g, 85%). ^{1}H NMR ($CDCl_3$, 300MHz): 3.58 (t (6.4 Hz), 2H); 2.40 (t (7.3 Hz), 2H); 2.06 (m, 2H); 1.45 ppm (s, 9H). ^{13}C NMR ($CDCl_3$, 75MHz): 172.5, 81.1, 44.8, 33.1, 28.6, 28.4 ppm.

Example 6

4-Iodo-butyric acid tert-butyl ester

Sodium iodide (70.0 g, 467 mmol) was added to tert-butyl ester of example 5 (22.0 g, 124 mmol) dissolved in THF (300 mL), and the resulting yellow suspension was refluxed overnight. The solvent was removed under reduced pressure, and the residue dissolved in EtOAc (200 mL). After washing successively with water and saturated NaCl solution the organic phase was dried over magnesium sulfate, and solvent removed to provide the title iodide as a yellow oil (31.4 g, 94%). ^{1}H NMR ($CDCl_3$, 300MHz): 3.23 (t (6.7 Hz), 2H); 2.34 (t (7.3 Hz), 2H); 2.07 (m, 2H); 1.40 ppm (s, 9H). ^{13}C NMR ($CDCl_3$, 75MHz): 173.2, 81.0, 44.6, 35.1, 28.9, 6.0 ppm.

Example 7

4-((2S)-Amino-2-carboxy-ethylsulfanyl)-butyric acid tert-butyl ester:

A suspension of cysteine (6.6 g, 55.5 mmol) in methanol (50 mL) was cooled to 0°C and degassed under a stream of argon for 5 minutes. On addition of 2M sodium hydroxide solution (55.5 mL, 111 mmol) the cysteine dissolved, and tert-butyl ester of example 6 (15.0 g, 55.5 mmol) was added immediately in one portion. Stirring was continued for a further 5 minutes, before adjustment of the pH to ~8 with 2 M HCl. The solvent was removed under reduced pressure, and the residue desalted by rpHPLC to provide the title amino acid as a white solid (14.2 g, 97%). ^{1}H NMR (d_6-DMSO, 300MHz): 3.4 to 3.1 (br s, water); 2.97 (dd (3.8, 14.3 Hz), 1H); 2.70 (dd (8.7, 14.2 Hz), 1H); 2.51 (t (7.4 Hz), 2H); 2.29 (t (7.2 Hz), 2H); 1.72 (m, 2H); 1.38 ppm (s, 9H). ^{13}C NMR (d_6-DMSO, 75MHz): 175.2, 172.6, 80.2, 53.9, 34.0, 33.2, 30.6, 28.1, 24.7 ppm.

Example 8

4-[(2S)-Carboxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-butyric acid tert-butyl ester :

$NaHCO_3$ (14 g, 170 mmol) and Fmoc-OSu (18.7 g, 55.5 mmol) were added to a solution of amino acid of example 7 (14.0 g, 53.0 mmol) dissolved in 1:1 THF water (300mL), and the resulting solution stirred for 2 hours. The solvent was removed under reduced pressure, and the residue suspended in EtOAc (300 mL), and washed successively with water, 1 M HCl, saturated $NaHCO_3$ solution and brine. The organic layer was dried over magnesium sulfate, and solvent removed under reduced pressure to yield a yellow oil which was purified by

rpHPLC to provide the title acid as a white solid (19.7 g, 76%). ^{1}H NMR (d_6-DMSO, 300MHz): 7.90 (d (7.5 Hz), 2H); 7.73 (d (7.71 Hz), 2H); 7.42 (t (7.2 Hz), 2H); 7.32 (t (6.6 Hz), 2H); 4.65 (d (5.3 Hz), 2H); 4.30 (m, 2H); 2.91 (dd (3.7, 14.2 Hz), 1H); 2.76 (dd (8.6, 14.2 Hz), 1H); 2.51 (t (7.3 Hz), 2H); 2.26 (t (7.2 Hz), 2H); 1.72 (m, 2H); 1.39 ppm (s, 9H). ^{13}C NMR (d_6-DMSO, 75MHz): 174.6, 172.1, 156.2, 144.2, 144.1, 128.0, 127.4, 125.6, 120.5, 79.6, 60.5, 54.5, 46.8, 34.0, 33.2, 30.8, 28.1, 24.8 ppm.

Example 9

4-[(2S)-Allyloxycarbonyl-2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-butyric acid tert-butyl ester :

Allyl bromide (6.23 g, 51.5 mmol) was added in one portion to a suspension of K_2CO_3 (27 g, 200 mmol) and compound of example 8 (25.0 g, 51.5 mmol) in DMF (200 mL). The resulting solution stirred for 10 minutes, and then the solvent was removed under reduced pressure. The resulting residue was dissolved in EtOAc (500 mL) and washed successively with water, 1 M HCl, saturated $NaHCO_3$ solution, and brine. The organic layer was dried over magnesium sulfate, and solvent removed under reduced pressure to provide the title allyl ester as a yellow oil (25.0g, 92%). ^{1}H NMR (d_6-DMSO, 300MHz): 7.89 (d (7.60 Hz), 2H); 7.72 (d (7.71 Hz), 2H); 7.41 (t (7.1 Hz), 2H); 7.32 (t (7.3 Hz), 2H); 5.88 (m, 1H); 5.31 (d (16.7 Hz), 1H); 5.20 (d (11.7 Hz), 1H); 4.59 (d (5.3 Hz), 2H); 4.25 (m, 4H); 2.88 (dd (3.8, 14.1 Hz), 1H); 2.77 (m, 1H); 2.53 (t (7.3 Hz), 2H); 2.27 (t (7.3 Hz), 2H); 1.72 (m, 2H); 1.38 ppm (s, 9H). ^{13}C NMR (d_6-DMSO, 75MHz): 172.1, 170.9. 156.3, 144.1, 141.1, 132.6, 128.0, 127.4, 125.6, 120.5, 118.1, 80.0, 66.2, 60.1, 54.5, 47.0, 34.0, 32.7, 31.0, 28.1, 24.9 ppm.

Example 10

4-[2-Allyloxycarbonyl-2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-butyric acid :

The ester of example 9 (25.0 g, 47.5 mmol) was stirred in 99:1 TFA:water (50 mL) for 2 hours. The solvent was removed under reduced pressure, and the residue purified by rpHPLC to provide the title acid as a white solid (19.5 g, 88%). ^{1}H NMR (d_6-DMSO, 300MHz): 7.89 (d (7.1 Hz), 2H); 7.72 (d (7.1 Hz),

2H); 7.42 (t (7.1 Hz), 2H); 7.33 (t (7.6 Hz), 2H); 5.90 (m, 1H); 5.30 (d (17.3 Hz), 1H); 5.19 (d (9.4 Hz), 1H); 4.59 (d (5.2 Hz), 2H); 4.28 (m, 4H); 2.89 (dd (4.9, 13.5 Hz), 1H); 2.79 (m, 1H); 2.52 (t (7.3 Hz), 2H); 2.29 (t (7.3 Hz), 2H); 1.73 ppm (m, 2H). ^{13}C NMR (d_6-DMSO, 75MHz): 174.4, 170.9, 156.3, 144.1, 141.1, 132.6, 128.0, 127.4, 125.6, 120.5, 118.4, 66.2, 60.1, 54.5, 47.0, 32.8, 32.7, 31.1, 24.7 ppm.

Example 11 Coupling to acid of example 10 to resin

Commercially available N-Fmoc hydroxylamine 2-chlorotrityl resin (0.77 mmol/g, 10 g, 7.7 mmol) was shaken gently with 1:1 piperidine:DMF (30 mL) over night, and then flow washed with DMF for 1 minute. In a separate flask HATU (3.0 g, 7.8 mmol) was added to a solution of acid of example 10 (3.7 g, 7.8 mmol) and DIPEA (5.3 mL, 31.2 mmol) dissolved in DMF (10 mL), and the resulting solution stirred gently for 5 minutes. The HATU activated acid was then added in one portion to the deprotected resin, and the resin was shaken gently for 1 hour. After washing the resin well with DMF, the resin loading was determined to be 0.46 mmol/g (70%) (LRMS *m/e* calc. for $C_{25}H_{29}N_2O_6S$ (MH^+) 485.6, obs. 485.1). The unreacted resin was then acylated by addition of a solution of acetic anhydride (842 mg, 7.8 mmol) and DIPEA (5.3 mL, 31.2 mmol) in DMF (20mL) with shaking for 2 minutes, followed by thorough washing with DMF.

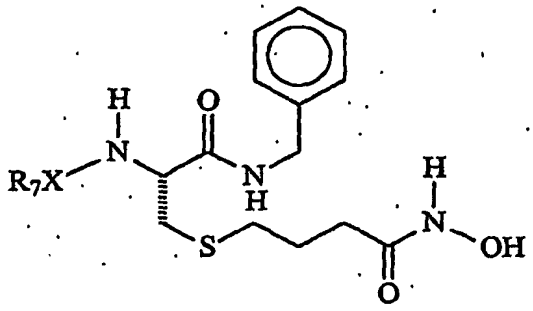
Example 12 Removal of the allyl protecting group

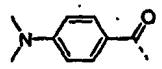
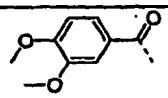
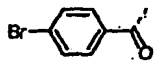
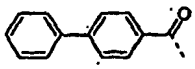
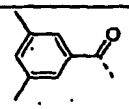
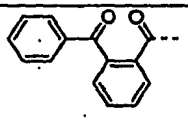
The resin of example 11 was flow washed with DCM for 2 minutes, and then shaken in DCM (30 mL) for a further 10 minutes. An argon stream was introduced, and the resin and DCM degassed for 5 minutes. DMBA (1.2 g, 7.9 mmol) was added, and bubbling continued for a further minute to ensure thorough mixing. $Pd(PPh_3)_4$ (270 mg, 0.23 mmol) was added to the resin, the flask wrapped in aluminum foil, and after a further 30 seconds of degassing the argon stream was removed, and the resin shaken gently for 1 hour. The resin was flow washed successively with DCM, DMF, and DCM, before drying under high vacuum. The resin loading was determined to be 0.45 mmol/g (LRMS *m/e* calc. for $C_{22}H_{25}N_2O_6S$ (MH^+) 445.5, obs. 445.2).

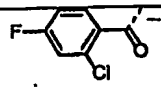
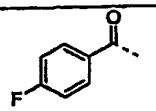
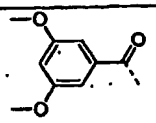
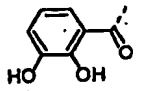
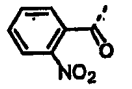
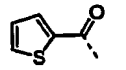
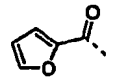
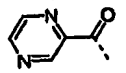
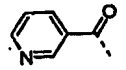
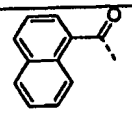
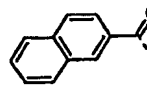
The product of example 12 was subjected to the general procedures outlined in examples 2-4 with variations made to the amine moiety used for coupling to the acid in example 2 and the acid moiety used for reaction in example 3 to produce the compounds given in tables 1, 2, 3 and 4. For example utilising benzylamine as the amine used according to the procedure in example 2 and by using a number of acids as the coupling moiety according to the general procedure of example 3 the compounds in table 1 were produced as examples 13-49. Similarly, by utilising 4-dimethylamino benzoic acid as the coupling moiety according to the general procedure of example 3 and varying the amine used according to the procedure in example 2 the compounds in table 2 were produced as examples 50-87

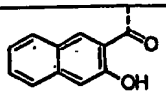
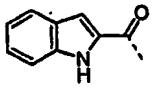
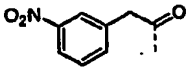
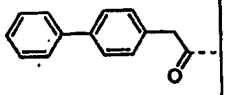
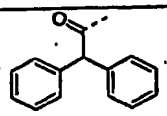
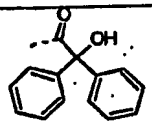
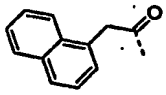
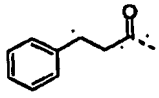
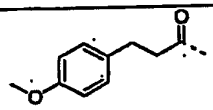
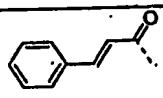
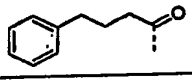
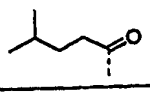
Following similar methodology using cinnamic acid as the coupling moiety according to the general procedure of example 3 and varying the amine used according to the procedure in example 2 the compounds in table 3 were produced as examples 88-93.

Table 1. HPLC Retention Time and HRMS Data for Compounds of Examples 13-48



Compound of Example	R_7-X	RpHPLC RT-Iso (min)	RT-grad (min)	HRMS (g/mol)	MS-theoretical
13		9.17	17.04	459.2051	459.2061
14		2.32	16.66	445.1909	445.1904
15		7.25	24.56	494.0742	494.0744
16		12.00	26.57	492.1987	492.1987
17		7.45	24.69	444.1989	444.1952
18		11.46	26.27	521.1745	521.1741

19		11.41	18.91	468.1143	468.1155
20		10.87	18.61	434.1528	434.1545
21		11.60	10.97	476.1867	476.1850
22		9.39	17.34	448.1538	448.1537
23		10.15	17.96	461.1504	461.1490
24		4.48	21.77	422.1211	422.1203
25		9.25	17.00	406.1419	406.1431
26		3.44	19.79	418.1574	418.1544
27		7.80	14.60	417.1593	417.1591
28		7.87	24.99	466.1816	466.1795
29		7.53	24.77	466.1791	466.1795

30		20.93	21.72	482.1759	482.1744
31		6.26	23.94	455.1744	455.1748
32		10.69	18.42	475.1672	475.1646
33		11.92	26.49	506.2143	506.2108
34		17.66	21.09	506.2098	506.2108
35		8.81	25.38	522.2088	522.2057
36		7.60	24.78	480.1955	480.1952
37		11.46	18.94	444.1950	444.1952
38		11.21	18.83	474.2058	474.2057
39		5.95	23.67	442.1830	442.1795
40		7.13	24.49	458.2127	458.2108
41		5.53	22.92	410.2127	410.2108

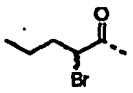
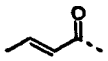
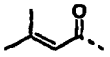
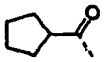
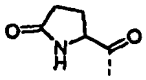
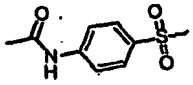
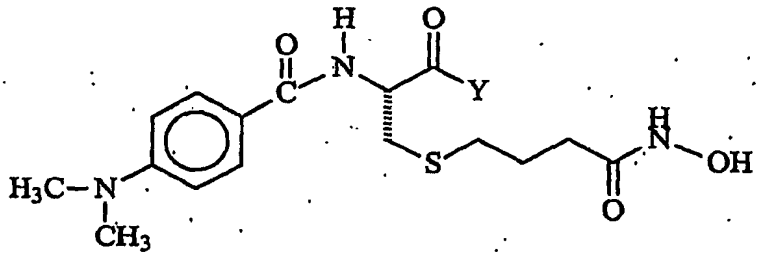
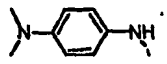
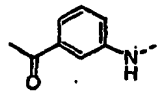
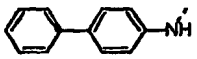
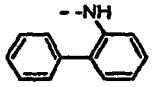
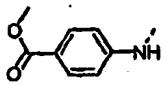
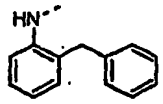
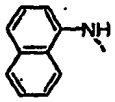
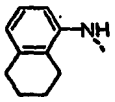
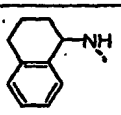
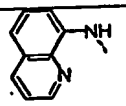
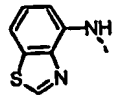
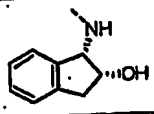
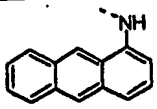
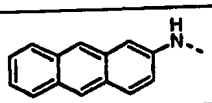
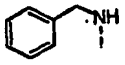
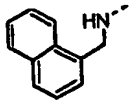
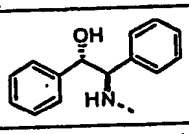
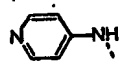
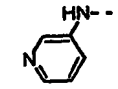
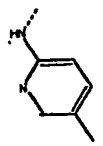
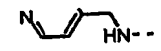
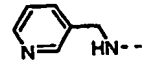
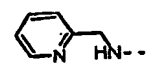
42		5.46	23.01	422.2131	422.2108
43		8.89	16.60	380.1638	380.1639
44		9.72	17.54	394.1790	394.1795
45		10.19	17.96	408.1949	408.1952
46		8.81	16.38	380.1640	380.1639
47		7.72	24.85	423.1701	423.1697
48		0.6	17.51	434.1388	434.1381

Table 2. HPLC Retention Time and HRMS Data for Compounds of Examples 50-87



Compound of Example	Y	RT-Iso (min)	RT-grad (min)	HRMS (g/mol)	MS-theoretical
50		7.66	15.36	488.2301	488.2326
51		8.30	18.23	487.2025	487.2010
52		8.83	25.38	521.2199	521.2217
53		9.75	21.61	521.2237	521.2217
54		9.98	20.16	503.1969	503.1959
55		12.01	20.05	535.2393	535.2374
56		10.56	18.97	495.2058	495.2061
57		10.87	19.75	499.2396	499.2374
58		9.25	20.55	499.2396	499.2374

59		9.77	17.95	496.2005	496.2013
60		11.10	19.18	502.1590	502.1577
61		8.85	19.98	501.2148	501.2166
62		10.88	23.21	545.2228	545.2217
63		12.64	24.48	545.2228	545.2217
64		9.15	20.57	459.2074	459.2061
65		10.27	22.44	549.2547	549.2530
66		11.06	23.10	509.2230	509.2217
67		9.71	21.45	565.2452	565.2479
68		7.56	15.14	446.1773	446.1791
69		7.76	16.03	446.1776	446.1791
70		7.67	15.65	460.2009	460.2013
71		7.38	14.34	460.2009	460.2013
72		8.01	17.79	460.2009	460.2013
73		7.46	14.58	460.2009	460.2013

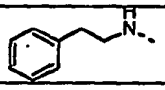
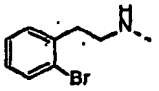
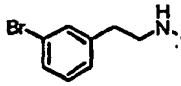
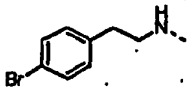
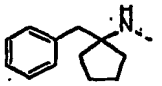
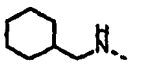
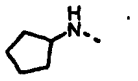
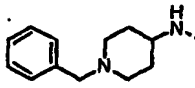
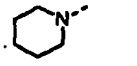
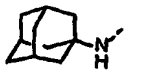
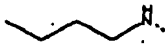
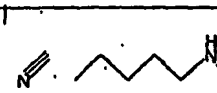
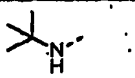
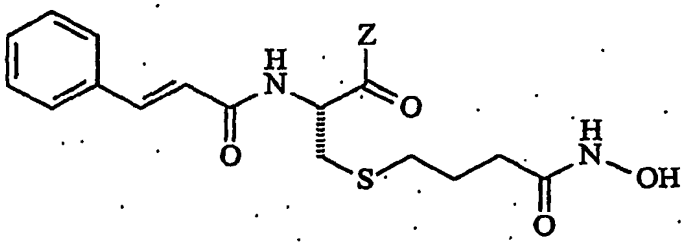
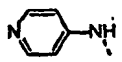
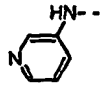
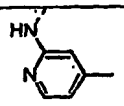
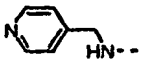
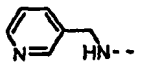
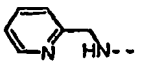
74		10.07	18.31	473.2227	473.2217
75		8.76	19.55	551.1312	551.1322
76		8.98	20.06	551.1315	551.1322
77		11.28	23.26	551.1305	551.1322
78		10.26	22.37	527.2697	527.2687
79		8.91	19.92	465.2521	465.2530
80		9.40	17.12	437.2206	437.2217
81		8.13	17.76	542.2802	542.2796
82		7.95	16.92	437.2199	437.2217
83		9.79	21.65	503.2699	503.1697
84		8.34	15.38	455.2344	455.2323
85		8.52	16.72	439.2347	439.2374
86		7.88	16.54	464.2339	464.2321
87		8.87	19.50	425.2194	425.2217

Table 3. HPLC Retention Time and HRMS Data for Compounds of Examples 88-93



5

Compound of Example	Z	RT-Iso (min)	RT-grad (min)	HRMS (g/mol)	MS-theoretical
88		8.21	18.84	429.1610	429.1591
89		7.95	17.10	429.1610	429.1591
90		8.10	18.02	443.1610	443.1591
91		7.82	17.63	443.1611	443.1591
92		7.73	15.47	443.1610	443.1591
93		8.03	18.09	443.1612	443.1591

Selected chemical data for a number of the compounds in tables 1 to 3 is given as follows:

Hydroxamic Acid of example 13 (R^7 = 4-Dimethylamino Benzoic Acid): ^{1}H NMR (d_6-DMSO, 500 MHz): 10.32 (s, 1H); 10.02 (s, 1H); 8.51 (t (5.9 Hz), 1H); 8.14 (d (8.3 Hz), 1H); 7.70 (d (8.7 Hz), 2H); 7.24 to 7.10 (m, 5H); 6.63 (d (8.7 Hz), 2H); 4.52 (m, 1H); 4.21 (d (5.9 Hz), 2H); 2.89 (s, 6H); 2.86 (obsc m (5.2 Hz)); 2.76 (dd (9.5, 13.5 Hz), 1H); 2.44 (m, 2H); 1.94 (t (7.5 Hz), 2H); 1.64 ppm (m, 2H). HRMS calc. for $C_{23}H_{31}N_4O_4S$ (MH^+): 459.206, Found 459.201.

Hydroxamic Acid of example 15 (R^7 = 4-Bromobenzoic Acid): ^{1}H NMR (d_6-DMSO, 500 MHz): 10.27 (s, 1H); 8.63 (d (7.9 Hz), 1H); 8.56 (t (5.5 Hz), 1H); 7.76 (d (8.7 Hz), 2H); 7.61 (d, 7.9 Hz), 2H); 7.25 to 7.15 (m, 5H); 4.54 (m, 1H); 4.21 (d (5.5 Hz), 2H); 2.90 (dd (4.8, 13.5 Hz), 1H); 2.75 (dd (9.5, 13.5 Hz), 1H); 2.45 (m, 2H); 1.93 (br t (7.1 Hz), 2H); 1.65 ppm (m, 2H). HRMS calc. for $C_{21}H_{25}BrN_3O_4S$ (MH^+): 494.074, Found 494.076.

Hydroxamic Acid of example 29 (R^7 = 2-Napthoic acid): ^{1}H NMR (d_6-DMSO, 500 MHz): 10.30 (s, 1H); 10.04 (s, 1H); 8.70 (d (7.9 Hz), 1H); 8.60 (t (6.3 Hz), 1H); 8.45 (s, 1H); 7.97 to 7.87 (m, 4H); 7.52 (m, 2H); 7.25 to 7.10 (m, 5H); 4.63 (m, 1H); 4.25 (d (5.5 Hz), 2H); 2.95 (dd (4.8, 13.5 Hz), 1H); 2.83 (ddd (1.6, 9.5, 13.5 Hz), 1H); 2.49 (m, 2H); 1.96 (m, 2H); 1.67 ppm (m, 2H). HRMS calc. for $C_{25}H_{28}N_3O_4S$ (MH^+): 466.179, Found 466.178.

Hydroxamic Acid of example 31 (R^7 = 1*H*-Indole-2-carboxylic acid): ^{1}H NMR (d_6-DMSO, 500 MHz): 11.52 (s, 1H); 10.28 (s, 1H); 8.61 (t (6.0 Hz), 1H); 8.53 (d (8.3 Hz), 1H); 7.55 (d (7.9 Hz), 1H); 7.34 (d (7.9 Hz), 1H); 7.25 to 7.20 (m, 6H); 7.10 (t (7.1 Hz), 1H); 6.96 (t (7.1 Hz), 1H); 4.60 (m, 1H); 4.24 (m, 2H); 2.91 (dd (5.5, 13.9 Hz), 1H); 2.77 (dd (9.5, 13.9 Hz), 1H); 2.47 (m, 2H); 1.95 (br t (6.7 Hz), 2H); 1.66 ppm (m, 2H). HRMS calc. for $C_{23}H_{27}N_4O_4S$ (MH^+): 455.175, Found 455.171.

Hydroxamic Acid of example 39 (R^7 = Cinnamic Acid): ^{1}H NMR (d_6-DMSO, 500 MHz): 10.29 (s, 1H); 8.62 (t (5.5 Hz), 1H); 8.32 (d (7.9 Hz), 1H); 7.47 (br d

(7.13 Hz), 2H); 7.37 to 7.10 (m, 9H); 6.71 (d (15.9 Hz), 1H); 4.52 (dd (7.9, 14.2 Hz), 1H); 4.22 (d (6.3 Hz), 2H); 2.80 (dd (6.3, 13.5 Hz), 1H); 2.65 (dd (7.9, 13.5 Hz), 1H); 2.45 (t (7.1 Hz), 2H); 1.96 (br t (7.9 Hz), 2H); 1.65 ppm (m, 2H). HRMS calc. for $C_{23}H_{28}N_3O_4S$ (MH^+): 442.179, Found 442.176.

Hydroxamic Acid of example 50 (NR_6XR_7 = 4-Dimethylamino benzylamine): ^1H NMR (d_6-DMSO, 500 MHz): 10.30 (s, 1H); 8.18 (d (7.9 Hz), 1H); 7.70 (d (8.7 Hz), 2H); 7.42 (br s, 2H); 6.84 (br s, 2H); 6.62 (d (8.7 Hz), 2H); 4.60 (m, 1H); 2.88 (s, 12H); 2.80 (m, 2H); 2.48 (m, 2H); 1.95 (m, 2H); 1.68 ppm (m, 2H). HRMS calc. for $C_{24}H_{34}N_5O_4S$ (MH^+): 488.2326, Found **488.2301**.

Hydroxamic Acid of example 52 (NR_6XR_7 = 4-Aminobiphenyl): ^1H NMR (d_6-DMSO, 500 MHz): 10.30 (s, 1H); 10.20 (s, 1H); 8.24 (d (7.1 Hz), 1H); 7.72 (d (8.7 Hz), 2H); 7.65 (d (8.7 Hz), 2H); 7.55 (m, 4H); 7.35 (t (7.9 Hz), 2H); 7.24 (t (7.9 Hz), 1H); 6.64 (d (8.7 Hz), 2H); 4.66 (dd (7.9, 14.3 Hz), 1H); 2.92 (obsc m (5.5 Hz)); 2.90 (s, 6H); 2.84 (dd (8.7, 13.5 Hz), 1H); 2.51 (t (7.1 Hz), 2H); 1.98 (m, 2H); 1.69 ppm (m, 2H). HRMS calc. for $C_{28}H_{33}N_4O_4S$ (MH^+): 521.2217, Found **521.2199**.

Hydroxamic Acid of example 56 (NR_6XR_7 = 8-Aminoquinoline): ^1H NMR (d_6-DMSO, 500 MHz): 10.54 (s, 1H); 10.29 (s, 1H); 8.71 (dd (1.6, 3.9 Hz), 1H); 8.67 (d (7.9 Hz), 1H); 8.56 (d (7.9 Hz), 1H); 8.32 (dd (1.6, 8.3 Hz), 1H); 7.77 (d (8.7 Hz), 2H); 7.60 (d (7.1 Hz), 1H); 7.52 (m, 2H); 6.70 (d (9.1 Hz), 2H); 4.75 (m, 1H); 3.12 (dd (4.8, 13.9 Hz), 1H); 2.92 (s, 6H); 2.88 (m, 1H); 2.47 (m, 2H); 1.97 (t (7.1 Hz), 2H); 1.69 ppm (m, 2H). HRMS calc. for $C_{25}H_{30}N_5O_4S$ (MH^+): 495.2061, Found 495.2058.

Hydroxamic Acid of example 64 (NR_6XR_7 = Benzyl Amine): ^1H NMR (d_6-DMSO, 500 MHz): 10.32 (s, 1H); 10.02 (s, 1H); 8.51 (t (5.9 Hz), 1H); 8.14 (d (8.3 Hz), 1H); 7.70 (d (8.7 Hz), 2H); 7.24 to 7.10 (m, 5H); 6.63 (d (8.7 Hz), 2H); 4.52 (m, 1H); 4.21 (d (5.9 Hz), 2H); 2.89 (s, 6H); 2.86 (*obsc* m (5.2 Hz)); 2.76 (dd (9.5, 13.5 Hz), 1H); 2.44 (m, 2H); 1.94 (t (7.5 Hz), 2H); 1.64 ppm (m, 2H). HRMS calc. for $C_{23}H_{31}N_4O_4S$ (MH^+): 459.2061, Found 459.2074.

Hydroxamic Acid of example 87 (NR_6XR_7 = ^{t}Butyl Amine): ^{1}H NMR (d_6-DMSO, 500 MHz): 10.28 (s, 1H); 10.00 (s, 1H); 7.90 (d (8.3 Hz), 1H); 7.66 (d (8.7 Hz), 2H); 7.50 (s, 1H); 6.62 (d (8.7 Hz), 2H); 4.44 (m, 1H); 2.89 (s, 6H); 2.77 (dd (5.2, 13.5 Hz), 1H); 2.70 (dd (8.7, 13.1 Hz), 1H); 2.45 (m, 2H); 1.94 (t (6.7 Hz), 2H); 1.64 ppm (m, 2H). HRMS calc. for $C_{20}H_{33}N_4O_4S$ (MH^+): 425.2217, Found 425.2194.

Biological data

The cytotoxicities of the compounds of the invention were determined by clonogenic survival of human cancer cells (MM96L, melanoma) and human normal cells (NFF, neonatal foreskin fibroblasts). Cells were incubated with the compounds at various concentrations of compound (0.01 – 10 μg/mL) for 24 hours, washed, and then grown for a further four days in the absence of hydroxamic acid before determining cell survival by cell count. The final readout involved staining with sulforhodamine B (SRB), a cost-effective method amenable to automation and high throughput analysis. At the technical level, "cell sensitivity" is often inferred from short term (1-2 day) observations such as apoptosis, which may not be a satisfactory model of clonogenic survival. Compounds were considered for further testing if they exhibited either potency (IC_{50} 200 nM) or selectivity (SI >5) in their killing of cancer cells over normal cells.

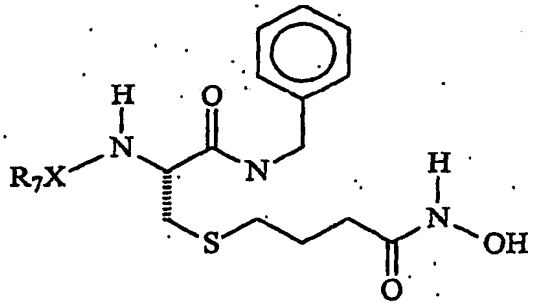
Cell Lines and Culture Medium. All cell lines used in this study have been described previously.[I] All cell lines were cultured in 10% heat-inactivated foetal calf serum (CSL, Australia) in RPMI 1640 medium supplemented with 100 U/mL penicillin, 100 g/mL streptomycin, and 3 mM HEPES at 5% CO_2, 99% humidity at 37°C. Primary human fibroblasts were obtained from neonatal foreskins and cultured in the above medium. Routine mycoplasma tests were performed using Hoechst stain[II] and were always negative.

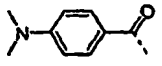
Cell Survival Assay. Cells were plated into 96-well microtitre plates at 5×10^3 cells / well, and allowed to adhere overnight. Compounds were added to culture medium at the indicated concentrations, and plates incubated in the

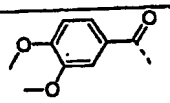
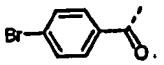
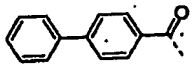
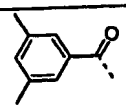
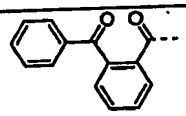
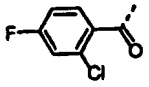
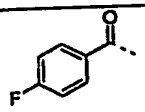
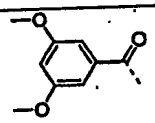
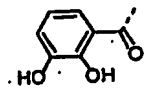
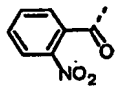
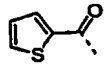
above conditions for 24 hours. Following this incubation period, compounds and media were removed, and replaced with fresh culture medium. Cells were then grown for a further 72 hours before assay using sulforhodamine B (SRB; Sigma, St. Louis, MO) as previously described.[iii] Briefly, the culture medium was removed from the 96-well microtitre plates and the plates washed twice with phosphate buffered saline (PBS), before the cells were fixed with methylated spirits for 15 minutes. The plates were then rinsed with tap water and the fixed cells stained with 50 µL / well of SRB solution (0.4% sulforhodamine B (w/v) in 1% (v/v) acetic acid) over a period of 1 hour. The SRB solution was then removed from the wells and the plates rapidly washed two times with 1% (v/v) acetic acid. Protein bound dye was then solubilised with the addition of 100 µL of 10 mM unbuffered Tris, and incubated for 15 min at 25°C. Plates were then read at 564 nm on a VERSA max tuneable microplate reader (Molecular Devices, Sunnyvale, CA).

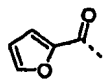
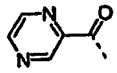
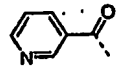
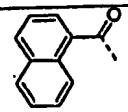
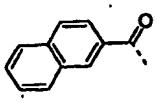
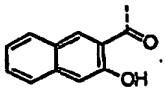
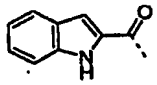
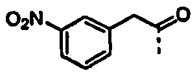
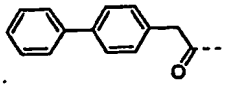
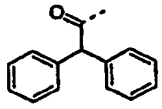
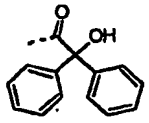
The results of the biological test results on each of the compounds is as given in the following tables.

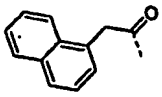
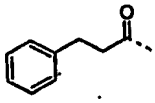
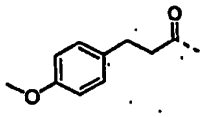
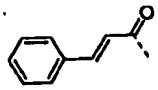
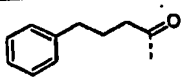
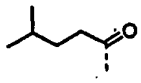
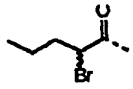
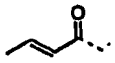
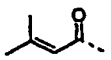
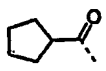
Table 4 Activity of Compounds of Examples 13-49



Compound of Example	R_7-X	Log $D_{7.0}$	IC_{50} NFF (µM)	IC_{50} MM96L (µM)	Selectivity
13		2.1	0.35±0.07	0.14±0.09	2.5

14		1.6	8.3±0.8	1.7±0.1	4.9
15		2.7	0.83±0.09	0.02±0.1	4.2
16		3.5	2.8±0.2	0.9±0.1	3.1
17		2.8	10.9±0.9	2.0±0.4	5.5
18		3.0	30±1	24±3	1.3
19		2.2	>100	28±3	>3
20		1.5	26±1	5.2±0.6	5.0
21		1.5	4.5±0.6	1.7±0.3	2.6
22		0.7	4.5±0.6	32±3	0.14
23		0.9	>100	10.6±0.1	>10
24		1.8	9±1	2.5±0.2	3.6

25		0.5	22±1	7.2±0.2	3.1
26		0.4	62±5	19±2	3.3
27		0.1	>100	12.8±0.8	>8
28		2.8	5.3±0.6	6.3±0.6	0.8
29		2.8	1.14±0.05	0.6±0.2	1.9
30		2.5	>100	13±2	>8
31		1.4	0.8±0.2	0.13±0.09	6.2
32		1.0	>100	12±1	>8
33		3.5	21±2	12±3	1.8
34		2.6	22±2	9.3±0.3	2.4
35		2.9	9.3±0.7	1.8±0.2	5.2

36		2.8	15±1	4.1±0.7	3.7
37		1.5	21±3	7.4±0.5	2.9
38		1.4	16±1	8±1	2.0
39		2.2	0.8±0.2	0.2±0.1	4.0
40		2.6	11±2	5±1	2.2
41		1.8	25±4	7±2	3.6
42		2.4	10±1	5±2	2.0
43		0.4	>100	11±0.7	>9
44		0.8	22±1	11±1	2.0
45		1.0	>100	21±3	>5
46		0	>100	21±2	>5
		0.9	9.6±0.9	4.4±0.3	2.2

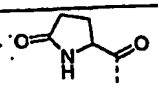
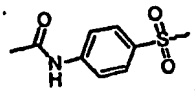
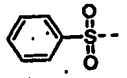
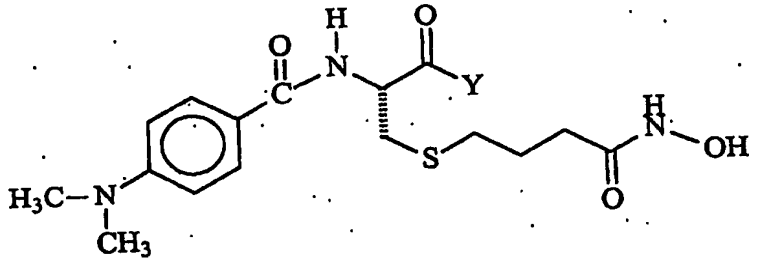
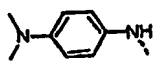
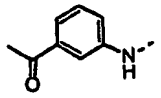
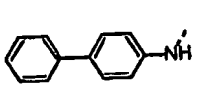
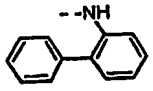
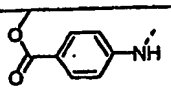
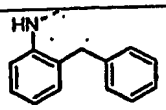
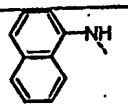
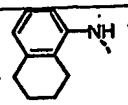
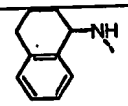
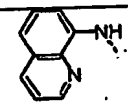
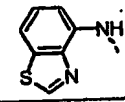
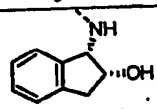
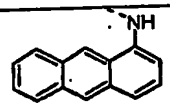
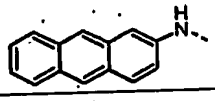
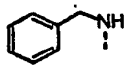
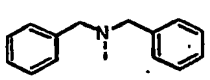
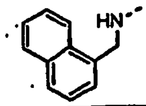
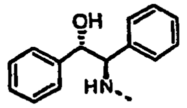
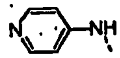
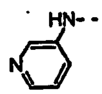
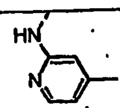
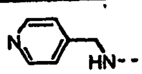
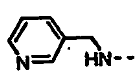
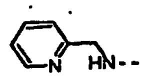
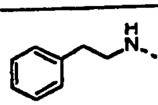
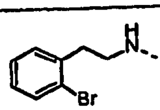
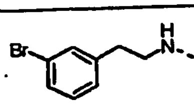
47					
48		0.6	>100	45±7	>2
49		1.7	>100	<100	>1

Table 5. Activity of Compounds of Examples 50-87



Compound of Example	Y	Log $D_{7.0}$	IC_{50} (μM)		Selectivity
			NFF	MM96L	
50		1.6	0.6±0.1	0.1±0.1	6.0
51		1.3	1.60±0.08	0.55±0.05	2.9
52		3.6	0.32±0.05	0.17±0.05	1.9
53		3.6	4.4±0.6	2.1±0.2	2.1

54		2.2	2.8±0.1	0.96±0.07	2.9
55		4.5	6.8±0.4	3.5±0.2	1.9
56		2.9	5.9±0.7	1.3±0.1	4.5
57		3.2	2.2±0.3	0.5±0.1	4.4
58		2.4	2.2±0.2	0.20±0.1	11
59		2.5	3.0±0.3	0.61±0.08	5.0
60		0.2	3.3±0.3	1.3±0.1	2.5
61		0.6	8.2±0.8	0.90±0.05	9.1
62		4.1	2.2±0.3	1.7±0.3	1.3
63		4.1	1.14±0.06	0.55±0.07	2.1
64		1.3	0.35±0.07	0.14±0.09	2.5
65		3.1	15.3±0.6	2.3±0.2	6.5

66		2.5	0.42±0.05	0.20±0.02	2.1
67		2.2	9±3	2.1±0.2	4.3
68		0.4	7.2±0.8	1.8±0.2	4.1
69		0.4	11±1	2.1±0.1	5.6
70		1.2	11.2±0.7	1.12±0.06	10.0
71		0.1	14±1	2.2±0.3	6.2
72		0.1	8.8±0.5	1.49±0.09	5.9
73		0.2	6.5±0.3	1.2±0.1	5.5
74		1.7	2.7±0.3	1.4±0.1	1.9
75		2.6	3.3±0.3	0.7±0.2	4.7
76		2.7	3.1±0.1	0.9±0.1	3.4
		2.6	2.6±0.3	1.0±0.1	2.6

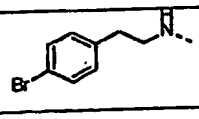
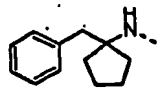
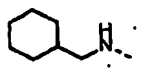
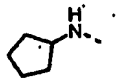
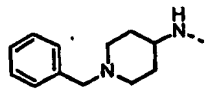
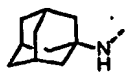
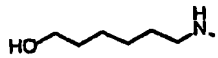
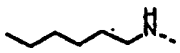
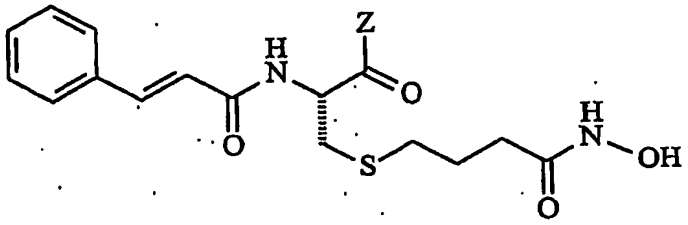
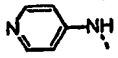
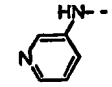
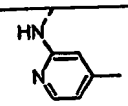
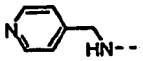
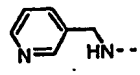
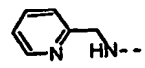
77					
78		3.1	4.2±0.5	0.6±0.1	7.0
79		2.1	3.6±0.2	0.51±0.09	7.1
80		1.9	2.6±0.3	1.5±0.1	1.7
81		0.4	1.28±0.08	1.5±0.2	0.9
82		0.9	20±2	4.9±0.3	4.2
83		2.3	7.1±0.5	1.48±0.08	4.8
84		0.8	12±1	1.8±0.2	6.7
85		1.7	7±1	0.85±0.04	8.0
86		1.4	6.0±0.8	1.3±0.2	4.5
87		0.9	3.5±0.5	0.7±0.2	5.0

Table 6. Activity of Compounds of Examples 88-93



Compound of Example	Z	Log $D_{7.0}$	IC50 (M)		Selectivity
			NFF	MM96L	
88		0.7	1.6±0.3	0.5±0.2	3.3
89		0.7	6.4±0.8	1.2±0.3	5.2
90		1.5	4.0±0.6	0.6±0.2	6.5
91		0.4	1.6±0.2	0.34±0.02	4.8
92		0.4	13.2±0.7	0.9±0.2	15.1
93		0.5	4.2±0.4	0.8±0.3	5.3

A number of the more active compounds were also tested for cytotoxicity and cytoselectivity against six other human cancer cell lines two melanoma (SkMel28, DO4), prostate (DU145), breast (MCF-7), and ovarian (JAM, CI-80-

13S). For comparison their results are also shown for MM96L and NFF cell lines. The results of these additional tests are given in table *7*.

TABLE 7. Cytotoxicity of Selected Compounds for Various Cancer Cell Lines

	Cell line[a] IC_{50} (μM)							
Compound	NFF	MM96L	SkMel	DO4	DU145	MCF7	JAM	C18013S
13	0.35 (7)	0.14 (9)	3.0 (3)	2.0 (3)	0.61 (4)	0.59 (5)	1.24 (6)	0.7 (2)
15	0.83 (9)	0.2 (1)	5.7 (4)	3.5 (2)	3.8 (4)	1.16 (2)	2.0 (2)	1.5 (3)
31	0.8 (2)	0.13 (9)	1.7 (2)	1.3 (4)	0.4 (4)	0.84 (9)	0.75 (8)	0.4 (3)
35	0.8 (2)	0.2 (1)	2.5 (1)	2.1 (3)	1.70 (3)	0.7 (2)	1.8 (1)	0.6 (5)
50	0.60	0.10	1.09	1.05	0.33	0.39	0.43	0.39
52	0.32	0.17	1.11	0.83	0.37	0.48	0.60	0.38
58	2.20	0.20	2.59	2.73	1.06	0.96	1.47	1.24
61	8.20	0.90	6.14	7.12	3.76	3.30	7.22	2.50
79	3.60	0.51	2.33	2.48	1.06	0.97	2.30	1.36

Histone Hyperacetylation. The more potent compounds from the series were tested for inhibition of histone deacetylase by monitoring the acetylation state of histone H4 using Triton-acetic acid-urea gel electrophoresis.

One set of results is shown in figure 1 for the compounds of examples 13 and 31, showing hyperacetylation of H4. It was not necessary to quantitate histone deacetylation because the compounds inhibit HDAC activity in both normal and cancer cells and has no impact on the cytoselectivity. The known HDAC inhibitor, TSA, included for comparison, showed similar levels of

hyperacetylation indicated by the mobility shift of histone H4. Clearly visible in untreated cells is the non-acetylated histone H4 (lane 1, arrow A). In the extracts from cells treated with 10 μg/ml of 13 and 31, histone H4 was observed in a variety of acetylation states, ranging from non-acetylated to tetra-acetylated. These results support the notion that this compound series inhibits HDACs.

Further results are outlined in figure 2 for just compounds **31** and **64**, showing hyperacetylation of H4. Once again, it was not necessary to quantitate histone deacetylation because the compounds inhibit HDAC activity in both normal and cancer cells and has no impact on the cytoselectivity. The known HDAC inhibitor, TSA, included for comparison, showed similar levels of hyperacetylation indicated by the mobility shift of histone H4. Clearly visible in untreated cells in the non-acetylated histone H4 (lane 1, arrow A). In the extracts from cells treated with 10 μg/ml of **31** and **64**, histone H4 was observed in a variety of acetylation states, ranging from non-acetylated to tetra-acetylated. These results support the notion that this compound series inhibits HDACs.

Induction of p21 Expression. It has been postulated that histone acetylation is associated with activation of gene transcription. It has been shown that the action of HDAC inhibitors on gene expression is somewhat selective, and does not lead to global deregulation of transcription as may be expected. In cells cultured with TSA, the expression of only 2% of genes was significantly altered, indicating a remarkable specificity. Possibly the best characterised gene to be induced following exposure to different HDAC inhibitors is that of the cyclin-dependent kinase inhibitor $p21^{WAF1/Cip1}$, which blocks cyclin-dependent kinase activity thereby causing cell-cycle arrest in G1. HDAC inhibitors are thought to act directly on the *CDKN1A* promoter rather than an upstream target. The HDAC inhibitor SAHA induces accumulation of acetylated histones in the chromatin associated with the *CDKN1A* gene, and this correlates with the observed increase in transcription. Sp-1 transcription factor binding sites in the promoter of *CDKN1A* are considered to be crucial for the observed induction,[34] and for a number of other targets. The capacity of novel compounds to induce

expression of the cyclin-dependent kinase inhibitor $p21^{WAF1/Cip1}$ (*CDKN1A*) was examined by semi-quantitative RT-PCR after 8/24 hours of treatment in MM96L and NFF cell types.

Cell Treatment and Total RNA Isolation. Cells were seeded in 25 cm^2 flasks in 10% heat-inactivated foetal calf serum (CSL, Melbourne, Australia) in RPMI 1640 medium supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 3 mM HEPES, and incubated at 5% CO_2, 99% humidity at 37°C for 16 hours before treatment. Cells were treated with 10 µg/mL of drug and RNA harvested at the indicated times following treatment. Total RNA was extracted from cells using the Qiagen RNeasy Kit as per manufacturer's instructions. RNA was analysed for sufficient quality by formamide agarose gel electrophoresis, and quantified by spectrophotometry.

p21 Expression. The semi-quantitative analysis of mRNA expression of $p21^{WAF1/Cip1}$ was carried out by RT-PCR. First strand synthesis was performed using 2 µg total RNA with 0.5 µg oligo $(dT)_{15}$ and 200 U SuperScript II (Invitrogen, Carlsbad, CA), at 42°C for 50 minutes in a final volume of 20 µL. Polymerase chain reaction was performed using 10 µL of a 1 in 10 dilution of the first strand cDNA, under standard conditions with the polymerase DyNAzyme (Finnzymes, Melbourne, Australia). Oligonucleotide primers and conditions used in the PCR were as follows: $p21^{WAF1/Cip1}$ F 5′- ATT AGC AGC GGA ACA AGG AGT CAG ACA T -3′, $p21^{WAF1/Cip1}$ R 5′- CTG TGA AAG ACA CAG AAC AGT ACA GGG T -3′ with initial denaturation at 94°C for 7 mins, 27 cycles of 94°C for 45 s, 60°C for 40 s and 72°C for 60 s, with the final extension for 5 minutes; GAPDH F 5′-GGC TCT CCA GAA CAT CAT CCC TGC-3′, GAPDH R 5′-GGG TGT CGC TGT TGA AGT CAG AGG-3′ with initial denaturation at 94°C for 7 minutes, 25 cycles of 94°C for 45 s, 62°C for 40 s and 72°C for 60 s, with the final extension for 5 minutes. Products were analysed by agarose gel electrophoresis, and visualised on a UV light box. Product intensity was determined to increase linearly with number of cycles and amount of mRNA used, by densiometric analysis using ImageQuaNT 4.2 software (Molecular Dynamics, Sunnyvale, CA). Quantitation of $p21^{WAF1/Cip1}$

induction was also performed by densitometric analysis using ImageQuaNT 4.2 software following normalisation to GAPDH product intensity.

Morphological Reversion. Cells were plated into 96-well microtitre plates at 5 $\times 10^3$ cells / well, and allowed to adhere overnight. Compounds were added to culture medium at the indicated concentrations, and plates incubated in the above conditions for 24 hours. Cells were then washed once with Hank's Balanced Salt Solution (HBSS; Gibco/Invitrogen, Grand Island, N.Y.), and fixed in 4% buffered formalin for 1 hour at room temperature. The fixed cells were then washed once further with HBSS and stained with 1% Crystal Violet in methanol for 5 minutes. Excess stain was removed by washing with tap water, before the microtitre plate being air dried at 37°C. Photographs were taken using a Leica DMIRB inverted microscope.

Finally, it will be appreciated that there may be other variations and modifications to the methods described herein that are also within the scope of the present invention.

DATED: 28 November 2003
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Attorneys for:
The University of Queensland

David B Fitzpatrick

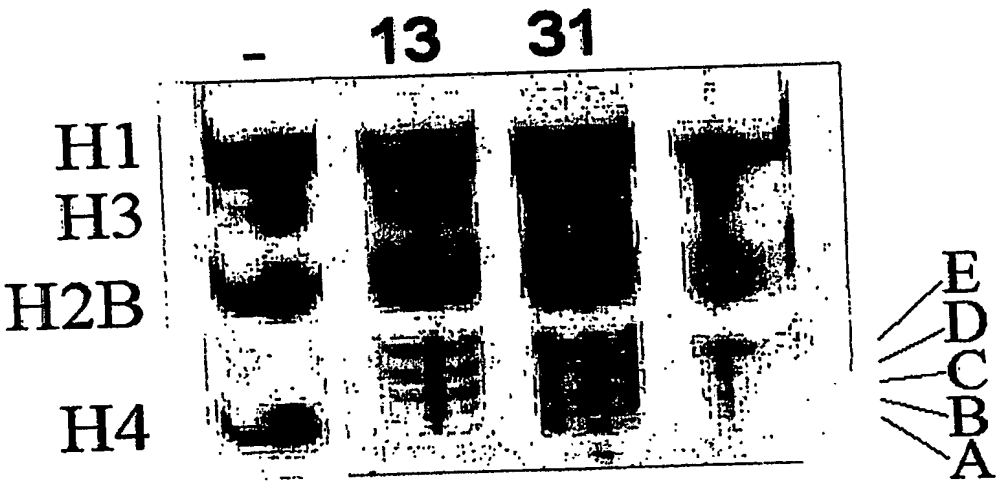

FIG 1

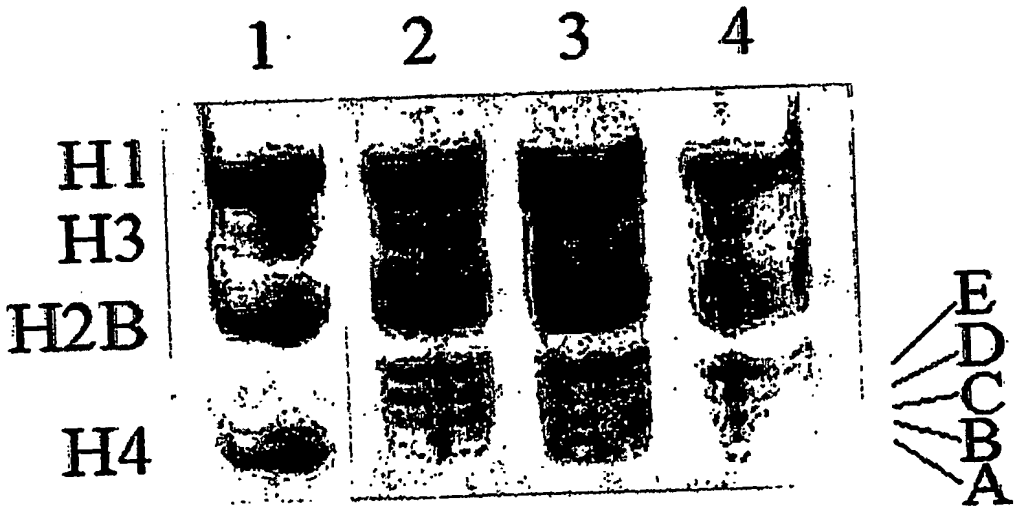

FIG 2

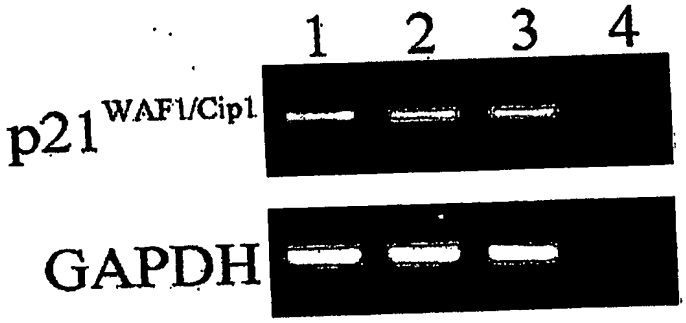

FIG 3

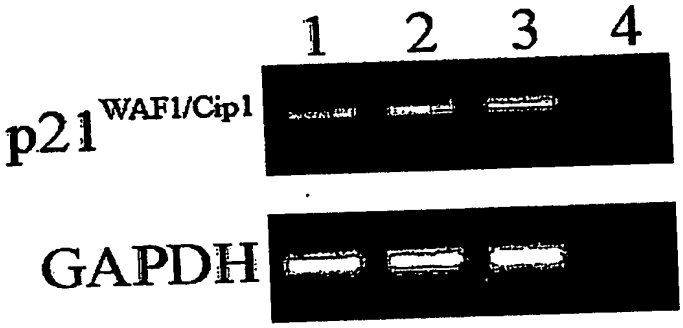

FIG 4

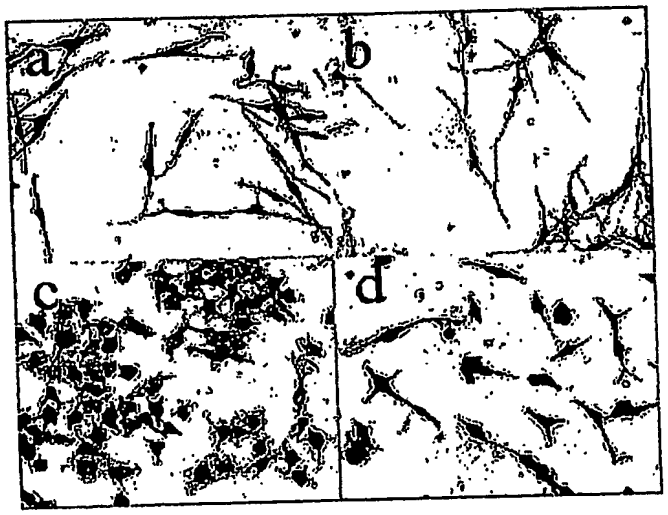

FIG 5

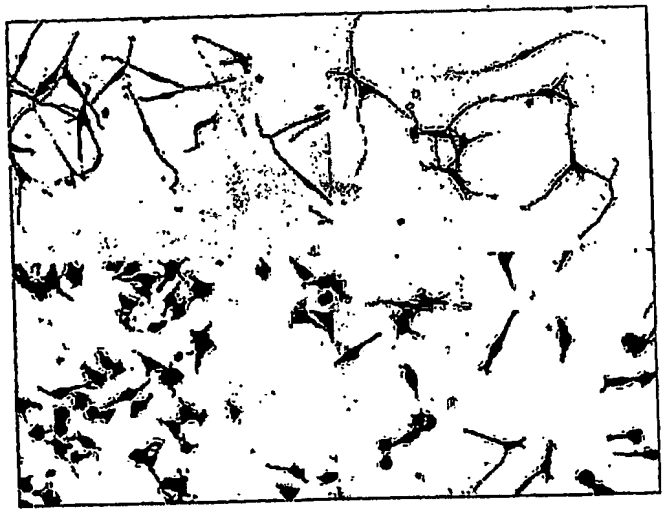

FIG 6

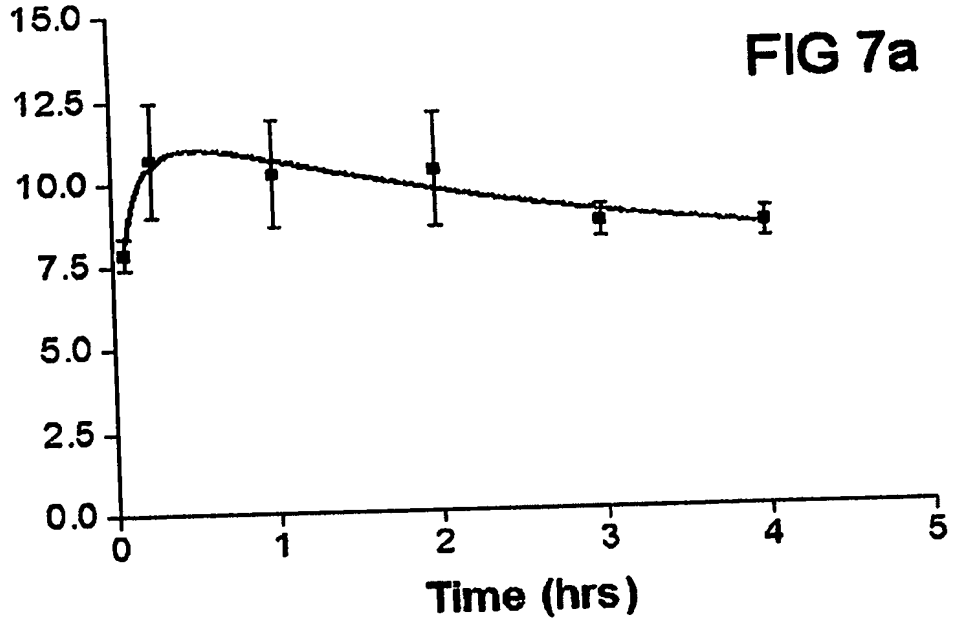
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0
1
2
3
4
5
Time (hrs)

FIG 7a

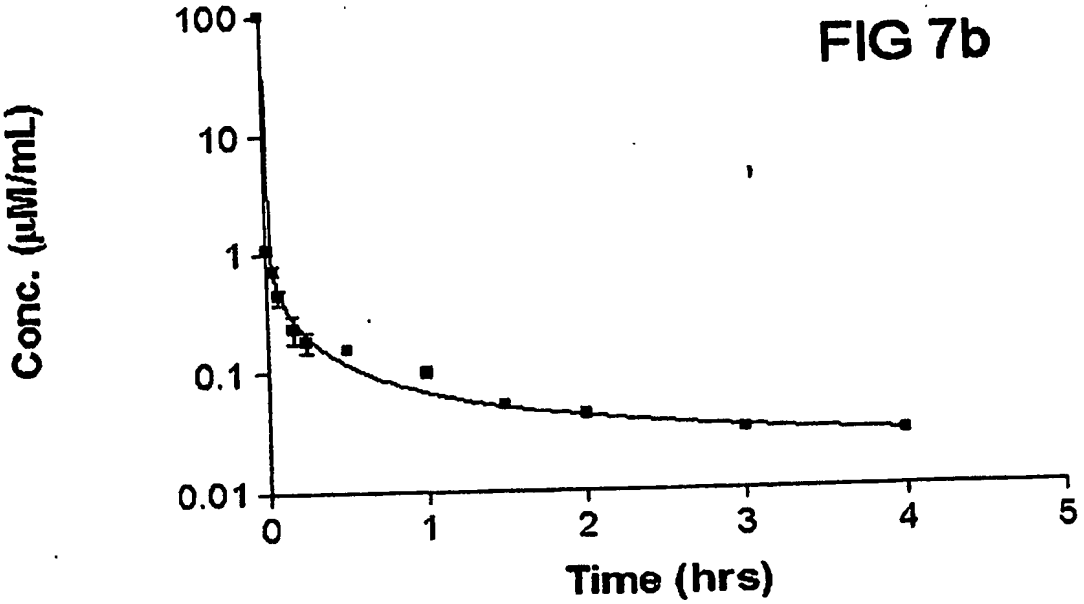
100
10
1
0.1
0.01
Conc. (µM/mL)
0
1
2
3
4
5
Time (hrs)

FIG 7b

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